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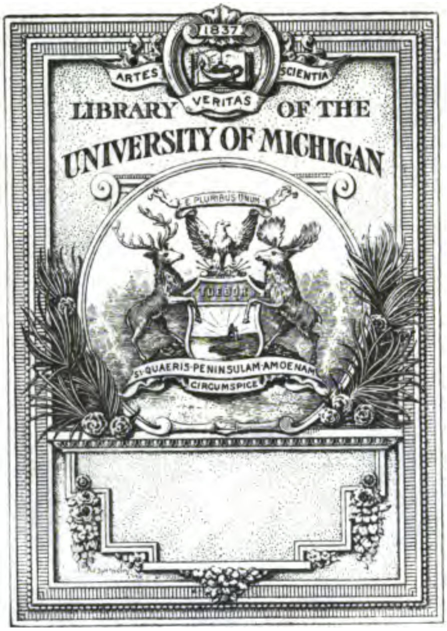
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THE DETECTION OF
POISONS AND STRONG DRUGS

AUTENRIETH — WARREN

THE DETECTION OF
POISONS AND STRONG DRUGS

INCLUDING

THE QUANTITATIVE ESTIMATION OF MEDICINAL PRINCIPLES IN CERTAIN CRUDE MATERIALS

BY

DR. WILHELM AUTENRIETH
PROFESSOR IN THE UNIVERSITY OF FREIBURG

AUTHORIZED TRANSLATION
FROM THE THIRD ENLARGED GERMAN EDITION

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Seventeen Illustrations

PHILADELPHIA
P. BLAKISTON'S SON & CO.
1012 WALNUT STREET
1909

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Reprinted with Additions, December, 1908

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LANCASTER, PA

0 59D/37.A9

AUTHOR'S PREFACE TO THE TRANSLATION.

In writing "Detection of Poisons" it was the author's intention to provide students of medicine and pharmacy with a concise laboratory-guide to toxicological analysis. Since the book is designed primarily for beginners, only the more important organic poisons which can be easily detected have been introduced. Many of these substances find frequent use in medicine, and others which are employed technically have often led to poisoning. Finally, to familiarize students of medicine and pharmacy with other common organic drugs, which are strong therapeutic agents rather than poisons, substances like quinine, phenacetine, salicylic acid, sulphonal, etc., have been included.

The last chapter deals with the quantitative estimation of certain active principles (alkaloids, glucosides, etc.) in crude materials used in medicine. It comprises also the estimation of caffeïne in coffee and tea, of theobromine in cacao-seeds and of nicotine in tobacco-leaves. The methods prescribed by the "German Pharmacopoeia" for estimating alkaloids have received especial attention, since the German pharmacist is obliged to determine whether or not his drugs comply with these requirements.

The detection of blood has been briefly considered, because students who perform the work described in these pages may at some future time be called upon as experts to examine stains for blood. Finally the biological detection of blood has been added to give the book completeness.

The author ventures to hope that his American and English colleagues may give the translation of his book the same kind reception accorded the German edition in his own country since its first appearance.

WILHELM AUTENRIETH.

FREIBURG IN BADEN.

260581

TRANSLATOR'S PREFACE.

In preparing Professor Autenrieth's book for English-speaking students of medicine and pharmacy, the purpose has been to follow the original as closely as is consistent with clearness. This book is, therefore, a translation and not an adaptation. The reader will come occasionally to methods based upon the "German Pharmacopoeia." The advisability of making these conform to the "U. S. Pharmacopoeia" was carefully considered. It was thought that those wishing to use these methods would in all likelihood have access to the "U. S. Pharmacopoeia," in which case they would be glad of the opportunity to compare the German with the American method. Consequently, these passages have been unchanged.

The translation follows the third edition in which the author presents the subject in a thoroughly revised form with many additions. The fifth chapter appears for the first time and contains directions for the quantitative estimation of alkaloids and other drugs in crude materials. While this new matter extends the scope of the book somewhat, it does not alter the general arrangement adopted in the earlier editions. The frequent references to the literature will be appreciated by those who may desire to read the original articles upon certain subjects. The brief comments of the author upon the fate, distribution and elimination of poisons in the animal body are a valuable feature of the last edition. The student learns what organs of the body should be examined for a poison in an actual analysis; what alterations it undergoes in metabolism; and in what form it is eliminated. The introduction of the biological methods used in detecting arsenic and human blood add much to the completeness of the work.

The writer takes this opportunity to thank Dr. R. W. Mills for the drawings used in illustrating the translation and Dr. W. H. Rush for reading the manuscript.

WILLIAM H. WARREN.

ST. LOUIS, MISSOURI,
March, 1905.

NOTE.

The writer has found it convenient to prepare for his own use a pamphlet in which the laboratory work in toxicology is divided into periods. These revised and enlarged laboratory directions have been added in the appendix. Frequent references to selected tests and full details for preparing material are given.

W. H. W.

CONTENTS.

PAGE.

AUTHOR'S PREFACE TO THE TRANSLATION v

TRANSLATOR'S PREFACE vii

INTRODUCTION 1

CHAPTER I. VOLATILE POISONS—GROUP I 3
- Yellow Phosphorus 4
- Hydrocyanic Acid 16
- Carbolic Acid 21
- Chloroform 28
- Chloral Hydrate 32
- Iodoform 33
- Nitrobenzene 34
- Aniline 35
- Ethyl Alcohol 37
- Acetone 40
- Carbon Disulphide 42
- Bitter Almond Water and Benzaldehyde 45

Synopsis of Group I 46

CHAPTER II. NON-VOLATILE, ORGANIC SUBSTANCES—GROUP II 49

A. Extraction of the Acid Solution with Ether 51
- Picrotoxin 54
- Colchicin 56
- Picric Acid 57
- Acetanilide 59
- Phenacetine 61
- Antipyrine 62
- Caffeïne 64
- Salicylic Acid 66

B. Extraction of the Alkaline Solution with Ether 68
- Coniïne 71

Nicotine 73
Aniline 74
Veratrine 74
Strychnine 76
Brucine 78
Atropine (Hyoscyamine) 80
Homatropine 82
Physostigmine 83
Cocaïne 84
Codeïne 87
Narcotine 89
Quinine 91
Caffeïne 94
Antipyrine 94
Pyramidone 95
C. Extraction of the Ammoniacal Solution with Ether and Chloroform 96
Apomorphine 97
Morphine 98
Narceïne 104
Appendix of Group II 106
Meconic Acid 106
Meconine 107
Digitalis Glucosides 108
Chloral Hydrate Solution as a Solvent 109
Ptomaïnes 111
Synopsis of Group II 112

CHAPTER III. METALLIC POISONS—GROUP III 117
Arsenic 124
Antimony 133
Tin 134
Mercury 135
Copper 136
Lead 136, 140
Bismuth 136
Cadmium 137

Zinc 137
Chromium 139
Silver 140
Barium 141
Synopsis of Group III.................................... 141
Biological Detection of Arsenic.................................... 142
Fate, Distribution and Elimination of Metals....... 145

CHAPTER IV. POISONS NOT IN THE THREE MAIN GROUPS. 151
Mineral Acids 151
Hydrochloric Acid 152
Nitric Acid 153
Sulphuric Acid 155
Alkalies 157
Potassium Chlorate 158
Oxalic Acid 161
Certain Organic Drugs 163
Santonin 164
Sulphonal 165
Trional 166
Blood 167
Carbon Monoxide in Blood.................................... 167
Blood-Stains 171
Biological Detection of Human Blood 179

CHAPTER V. QUANTITATIVE ESTIMATION OF ALKALOIDS AND OTHER DRUGS IN CRUDE MATERIALS 182
Estimation of Cantharidin in Spanish Flies........ 182
Estimation of Alkaloid in Cinchona Bark......... 183
Estimation of Alkaloid in Cinchona Extracts...... 185
Estimation of Caffeïne in Coffee, Tea, Cola Nuts, Etc. 185
Estimation of Alkaloid in Ipecac.................. 192
Estimation of Nicotine in Tobacco................ 194
Estimation of Hydrastine in Fluid Extract of Hydrastis 196
Estimation of Morphine in Opium................ 198
Estimation of Santonin in Wormseed............. 200

Estimation of Alkaloid in Nux Vomica............ 203
Estimation of Theobromine in Cacao-Seeds........ 205
Estimation of Alkaloid in Certain Officinal Extracts. 207
    Extract of Belladonna 207
    Extract of Hyoscyamus 208
    Extract of Nux Vomica 208
    Tincture of Nux Vomica 209
Estimation of Alkaloid in Pomegranate Bark 210
Estimation of Alkaloid in Aconite Root 210

APPENDIX 212
    A. General Alkaloidal Reagents.............. 212
    B. Other Reagents and Solutions............. 215
    C. Outline of a Laboratory Course........... 217

INDEX 261

INTRODUCTION.

Nearly all the ordinary poisons and drugs may be arranged in three groups. This classification, based upon the chemical behavior of these substances during isolation from mixtures for identification, is as follows:

Group I. This group includes volatile substances which distil undecomposed from an acid solution with steam. Yellow phosphorus, hydrocyanic acid, carbolic acid, chloroform, iodoform, aniline, nitrobenzene, carbon disulphide, etc., are the principal members of this group.

Group II. This group includes non-volatile, organic substances, which do not distil from an acid solution with steam. They may be extracted, however, from extraneous matter with hot alcohol containing tartaric acid. Alkaloids, many glucosides and bitter principles, as well as certain synthetic organic drugs like acetanilide, phenacetine, antipyrine, pyramidone, sulphonal, etc., are comprised in this group.

Group III. This group includes all poisonous metals.

Accordingly, in toxicological analysis poisons are divided into three groups, each having its own special method of procedure. A few poisons like mineral acids, caustic alkalies, oxalic acid and potassium chlorate cannot be placed conveniently in these three groups, owing to differences in solubility and other peculiarities. Special tests for such substances must be made with a separate portion of material. Chapter IV contains a description of the methods used in identifying these substances.

The material must be uniformly mixed and divided into three or four approximately equal portions, unless the analysis is to be limited to the detection of a single, well-defined substance. One portion is tested for non-volatile, organic substances (Chapter II). The second portion is examined for volatile poisons (Chapter I), and the residue from this portion is used in testing for poisonous metals (Chapter III). The third portion is tested for substances considered in Chapter IV. The fourth portion is held in reserve, in case additional material is needed to verify a doubtful result, or to replace a portion accidentally lost during analysis.

Occasionally, it is advisable to depart from the general procedure, and follow a special method, especially in detecting a single poison, or in estimating it quantitatively. For instance, pure ether would not be the best solvent to use in extracting strychnine quantitatively from an alkaline, aqueous solution. A mixture of ether and chloroform, or better pure chloroform, would be preferable, since strychnine is more soluble in this solvent than in pure ether. Chloroform also would be used in the quantitative extraction of caffeïne or antipyrine. When only a small quantity of material is available for the analysis, tests for all three groups of poisons may be made with the same portion. In this case, after removal of volatile poisons (Chapter I), the residue should be divided into two unequal portions. The larger portion must be tested for non-volatile, organic poisons (Chapter II). The smaller portion, together with the residue left after extracting non-volatile, organic poisons, must be tested for poisonous metals (Chapter III). It is advisable, however, also to reserve a portion of material for any contingencies.

Organs of the human body like liver, kidneys, spleen, heart, brain, stomach or intestines with contents, must be cut into small pieces and then finely chopped before being examined chemically. An organ should be cut first into small pieces with sharp, clean scissors, and then minced with a chopping-knife in a new, wooden bowl. Material may be held with nickel-plated tongs, while being cut with scissors.

CHAPTER I.

VOLATILE POISONS[1]—GROUP I.

Members of this group are volatile when an acid solution is distilled with steam. The distillate may contain:

Yellow Phosphorus
Hydrocyanic Acid
Carbolic Acid
Chloroform
Chloral Hydrate
Iodoform
Nitrobenzene
Aniline
Ethyl Alcohol
Acetone
Carbon Disulphide
Benzaldehyde
Bitter Almond Water.

Procedure.—The first requisite in toxicological analysis is uniform material. The character of the substance to be examined will determine whether it must be ground or chopped. Put a portion of material into a large, round-bottom flask and add enough water to permit free distillation. Add tartaric acid until the solution has a strong acid reaction, after being thoroughly shaken. Usually 20 to 30 drops of 10 per cent. tartaric acid solution are required. In examining material of animal origin, such as a stomach and contents, it is not necessary to add much water because a sufficient quantity is usually present. In such a case stir the material with a small quantity of water acidified with tartaric or dilute sulphuric acid and then distil.

---

[1] Laboratory practice in detecting poisons may be given by mixing small quantities (from 0.03 to 0.05 or 0.1 grams) of a poison with dry bread or biscuit crumbs. Finely chopped organs (liver, kidney, spleen, etc.), sausage-meat, beer or milk may be used. Drugs like morphine, codeïne, quinine, acetanilide, phenacetine, antipyrine, caffeïne, santonin, sulphonal, calomel, tartar emetic, subnitrate of bismuth, etc., may be mixed with powdered cane- or milk-sugar. The last kind of practice is especially suitable for students of pharmacy.

YELLOW PHOSPHORUS.[1]

In testing for yellow phosphorus distil according to the method of Mitscherlich. Arrange the apparatus as shown in Fig. 1. Support the condenser in a vertical position and connect the upper end with the flask by a glass tube about 8 mm. in internal diameter. This tube has two right-angle bends and each end passes through a cork. Have condenser and tube scrupulously clean to avoid interference with the phosphorescence.

Have the flask at most not more than one-third full. This precaution is necessary because many materials, containing proteïn substances like albumin, albumose, etc., and starchy matter, cause more or less foaming which is liable to carry over solid matter into the receiver. Use for the receiver an Erlenmeyer flask containing a small quantity of water (3 to 5 c.c.) into which the end of the condenser dips. This precaution prevents loss of easily volatile substances like hydrocyanic acid and chloroform. Heat the flask upon a wire-gauze or sand-bath and bring its contents to boiling by raising the temperature gradually. There is some danger of burning or carbonizing organic matter on the bottom of the flask, if heat be applied too strongly or rapidly. When boiling begins, make the room as dark as possible and watch for phosphorescence in the tube and condenser. When this is distinctly visible, the presence of yellow phosphorus in the material is established. Phosphorescence, during distillation with steam, is often the only sure and unquestionable test for the poisonous modification of phosphorus. Should phosphorescence not appear immediately, distillation must be continued for some time, because certain substances like ethyl alcohol, ether and turpentine, either prevent the phenomenon entirely, or seriously retard it. Considerable carbolic acid or chloral hydrate may have the same effect. Phosphorescence, however, often appears when these substances have passed over. But even if prolonged distillation fail to give

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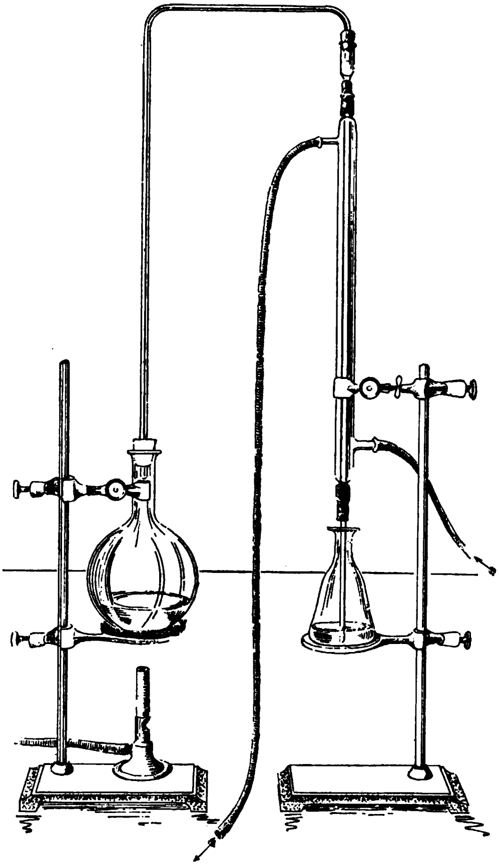
[1] It is advisable to make Scherer's test for phosphorus (page 6) before setting up the Mitscherlich apparatus.

FIG. 1.



Mitscherlich Apparatus..

a positive result, this must not be accepted as absolute proof of the absence of phosphorus, until other tests have been made. Whatever be the result, mix a portion of the distillate with an excess of saturated chlorine water and evaporate the mixture to dryness upon the water-bath. When phosphorus is present, it always imparts a strong odor to the distillate. Small drops of phosphorus appear if the quantity be large, and the solution contains phosphorous acid. Dissolve the residue from evaporation in about 5 c.c. of water and test it in two separate portions for phosphoric acid.

1. **Ammonium Molybdate Test.**—Acidify the solution with a few drops of concentrated nitric acid. Add an equal volume of ammonium molybdate solution ($((H_4N)_2MoO_4)$) and warm to about 40°.[1] Phosphoric acid precipitates yellow ammonium phospho-molybdate.

2. **Ammonium Magnesium Phosphate Test.**—Add magnesia mixture[2] to the second portion. Phosphoric acid gives a white, crystalline precipitate of ammonium magnesium phosphate ($((H_4N)MgPO_4.6H_2O)$). Vigorous shaking favors the precipitation. When only traces of phosphoric acid are present, long standing is necessary before the precipitate appears. Always examine the precipitate with the microscope. It should consist of well-formed crystals or at least should be crystalline. These crystals are transparent, acicular prisms (Fig. 2).

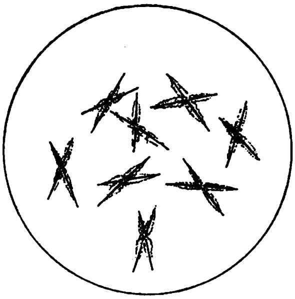
Scherer's Phosphorus Test.—This preliminary test for phosphorus should always precede the distillation. It is based upon the fact that moist phosphorus vapor and silver nitrate form phosphide of silver (Ag_3P), metallic silver and phosphoric acid. Put some of the original material into a small Erlenmeyer flask. Suspend in the mouth of the flask two strips of paper, one of which has been moistened with silver nitrate solution and the other with lead acetate solution. Warm gently upon the water-bath (40 to 50°). If the silver and not the lead paper be darkened, only yellow phosphorus is present. If both papers be darkened, hydrogen sulphide also is present. In the latter case yellow phosphorus may be present with hydrogen sulphide. Under the same conditions, it is also true that formaldehyde (H.CHO) and formic acid (H.COOH) can darken the silver paper.

---

[1] Temperature in this book is expressed in Centigrade degrees. Tr.

[2] Magnesia mixture is a clear, aqueous solution prepared by mixing equal volumes of solutions of magnesium chloride, ammonium chloride and ammonium hydroxide (about 10 per cent.). It contains the readily soluble double chloride of ammonium and magnesium which is not decomposed by ammonium hydroxide. This reagent is prepared as needed and should be perfectly clear and colorless.

FIG. 2.



Ammonium Magnesium Phosphate Crystals.

Scherer's test is evidently more valuable as proof of the absence than of the presence of free phosphorus. It is an excellent exploratory test, because it excludes phosphorus when the silver paper is unchanged.

Remarks Upon the Mitscherlich Test.—A. Hilger and H. Nattermann[1] state that 0.00006 grams of yellow phosphorus is the smallest quantity that can be detected by the Mitscherlich method. When 200 c.c. of water containing 0.0003 grams of phosphorus were distilled, there was brilliant phosphorescence lasting 5 minutes. The degree of dilution seems to have no effect upon the result, at least not within limits which occur in practice. Hydrogen sulphide which is always present in putrefying animal matter has no apparent effect upon phosphorescence. Free phosphorus can be detected in putrid organic matter even after the lapse of considerable time. Putrefactive and digestive processes appear to prevent oxidation of phosphorus. Dragendorff[1] states that he detected phosphorus in an exhumed body several weeks after death. Neumann found free phosphorus in a human body fourteen days and Elvers eight weeks after death.

---

[1] *Forschungsberichte über Lebensmittel und ihre Beziehungen zur Hygiene,* etc., 4, 241–258 (1897).

When an acidified, aqueous solution is distilled in the Mitscherlich apparatus, the residue in the flask always contains phosphoric, phosphorous and hypophosphorous acids and red phosphorus. In the distillation of a solution containing 0.0644 grams of phosphorus, only 71.33 per cent. of phosphorus passed into the distillate and the residue contained:

Phosphorus	as phosphoric acid	(H_3PO_4)	18.93	per cent.
"	" phosphorous acid	(H_3PO_3)	2.15	"
"	" hypophosphorous acid	(H_3PO_2)	4.27	"
"	" red phosphorus		2.98	"
			28.33	"

Oxidation of phosphorus may be prevented by conducting the distillation in a current of carbon dioxide.

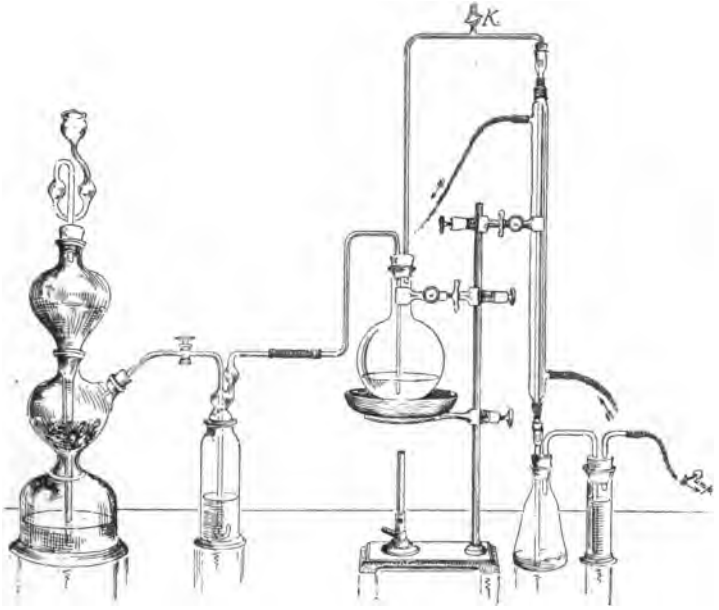
Quantitative Determination of Phosphorus (Mitscherlich-Scherer).—Acidify a weighed portion of material with dilute sulphuric acid and add a small quantity of ferrous sulphate. Distil in a gentle stream of carbon dioxide, using a large flask fitted with a stopper having two holes. Before applying heat, expel air completely from the apparatus by carbon dioxide. Use for the receiver a flask fitted with a stopper having two holes. Pass the end of the condenser through one hole and connect the other with a U-tube containing silver nitrate solution. Evaporate the distillate upon the water-bath with strong bromine water, or with concentrated nitric acid, to oxidize phosphorus or any phosphorous acid which has been formed. Dissolve the residue in a small quantity of water and precipitate phosphoric acid with magnesia mixture. Weigh the precipitate as magnesium pyrophosphate. Heat the contents of the U-tube with concentrated nitric acid. Precipitate silver as silver chloride and filter. Concentrate the filtrate by evaporation and pre-

[1] *Die gerichtlich-chemische Ermittelung von Giften,* III edition, 99.

cipitate phosphoric acid with magnesia mixture as before. Combine this precipitate with the other. In distillation, some phosphorus separates as globules in the first receiver and any, carried over by carbon dioxide as vapor, will be retained by silver nitrate solution. Since distillation of phosphorus by steam is very slow, the process should be carried on 2 or 3 hours.

A. Hilger and H. Nattermann (loc. cit.) recommend the form of apparatus shown in Fig. 3 for the quantitative determination of phosphorus. By opening stop-cock K and allowing air to come into contact with phosphorus vapor, it is possible to show the characteristic phosphorescence.

FIG. 3.



Hilger-Nattermann Apparatus.

Test for Phosphorus and Phosphorous Acid (Blondlot[1] and Dussard[2]).—It is often necessary, when the Mitscherlich method fails to show phosphorus, to test for phosphorous acid. This is the first product in the oxidation of phosphorus and is formed easily. The Blondlot-Dussard method shows the slightest trace of phosphorous and hypophosphorous acids as well as yellow phosphorus. The test is based upon the fact that hydrogen phosphide (phosphine), or hydrogen charged with phosphorus vapor, burns with a strong green flame which can easily be seen in a dark room. The green color is more distinct when a porcelain dish or plate is depressed upon the flame. Nascent hydrogen will reduce phosphorous and hypophosphorous acids and silver phosphide (Ag_3P) to phosphine (PH_3) but not orthophosphoric acid and its salts. Organic substances of animal origin may entirely prevent, or at least seriously hinder, the appearance of the green flame. This may be obviated by first passing the gas, which may contain phosphine, into silver nitrate solution. Examine in the Blondlot apparatus the black precipitate which may consist of silver phosphide.

Procedure.—Generate hydrogen in a large flask from phosphorus-free zinc and dilute sulphuric acid. Introduce into this flask some of the original material; or, in case a test is to be made for phosphorous acid alone, the filtered aqueous extract of the material; or the filtrate from the residue left after distillation by the Mitscherlich method. Pass the gas for some time (1 or 2 hours) through silver nitrate solution. Collect the black precipitate upon a filter free from ash. Wash well and examine in the Blondlot apparatus. A black precipitate does not necessarily prove the presence of phosphine, because the hydrides of arsenic (arsine) and antimony (stibine), hydrogen sulphide and several reducing substances give black precipitates with silver nitrate solution.

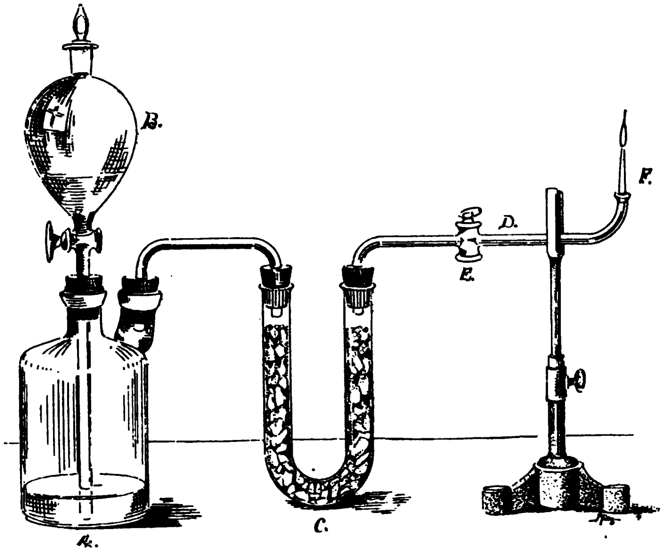
---

[1] Blondlot, *Journal de Pharmacie et de Chimie*, 3, T. 40, 25.

[2] Dussard, *Comptes rendus*, T. 43, 1126.

Generate hydrogen in flask A (Fig. 4) from pure zinc and dilute sulphuric acid. Fill U-tube C with pieces of pumice-stone saturated with concentrated potassium hydroxide solution. This will absorb any hydrogen sulphide. Use hard glass for tube D and have the tip F of platinum. The part marked E is a glass stop-cock or screw-tap. Reservoir B serves to hold liquid from A.

FIG. 4.



Blondlot Apparatus.

When hydrogen has completely expelled air, close stop-cock E. The pressure will force liquid from A into B. Open stop-cock E and light the gas at tip F. Regulate the flow so that the gas burns with a small flame. Test the hydrogen for phosphorus by darkening the room and noting if there be any green color when a porcelain dish is depressed upon the flame. If this test be negative, the apparatus is ready for use.

Wash into B the well-washed, black precipitate obtained from silver nitrate solution. Avoid loss while transferring the precipitate from the paper to the gas-flask. If the precipitate contain silver phosphide, a green cone will soon appear upon the hydrogen flame and a porcelain dish depressed upon the flame will produce a beautiful, emerald-green glow.

Hilger-Nattermann[1] Modification of Blondlot-Dussard Method.—Fit a 100 c.c. flask with a stopper having three holes, through two of which pass right-angle tubes ending below the stopper. Hydrogen from a Kipp generator enters the flask by one tube and escapes by the other. Connect the latter with a U-tube containing pieces of pumice-stone saturated with concentrated potassium hydroxide solution. Attach the tube with platinum tip to the U-tube. Pass a separating funnel through the third hole to the bottom of the flask. Cut into small pieces the paper holding the precipitate to be examined for phosphorus, and put these into the flask, which contains pieces of phosphorus-free zinc and enough water to seal the end of the separating funnel. Pass hydrogen through the apparatus and make sure that the flame is perfectly colorless[2] by the test already described. The surest test of the purity of zinc is afforded by a spectroscopic examination of the hydrogen flame. The spectrum of hydrogen from perfectly pure, commercial zinc consists of a single orange line in place of the yellow sodium line. Three green lines to the right of D show the slightest trace of phosphorus. The shade of two of these lines is deeper than that of the third. If this test show that zinc and sulphuric acid are pure, allow a few c.c. of dilute sulphuric acid (1 : 5) to run from the separating funnel into the flask. The flame is tinged green, when phosphorus is present, but sometimes the color does not appear for a long time. A spectroscopic examination of this flame must always be made.

---

[1] *Forschungsberichte über Lebensmittel und ihre Beziehungen zur Hygiene,* etc., 4, 241–258 (1897).

[2] It is difficult to get zinc which stands this test.

The Mitscherlich method affords a distillate especially suitable for the Blondlot-Dussard test. If this impart a green color to the hydrogen flame, there can be no question about the presence of phosphorus.

Should phosphorus not appear, it may be necessary to test the original material (for example, organs of the body) for phosphorous acid, the first oxidation product of phosphorus. Introduce some of the material into a flask where hydrogen is being generated and pass the gas into silver nitrate solution. Collect silver phosphide upon a filter and test it in the Blondlot apparatus. The reduction of phosphorous acid to phosphine takes place very slowly, even a few milligrams requiring the action of nascent hydrogen for 10 to 14 days. And even then only a small part (one-fifth to one-tenth) of the phosphorus appears in the receiver.[1] Moreover, careful manipulation is necessary, because silver phosphide is quite unstable. Water decomposes this substance into metallic silver and phosphorous acid, and the nitric acid present further oxidizes the latter, in part, to phosphoric acid. For this reason, Hilger and Nattermann recommend an examination of the precipitate (Ag_3P ?) and solution when reduction has been in progress two days.

Distillation by the Mitscherlich method, of material containing phosphorus, always produces phosphorous acid in the distillate. The Blondlot-Dussard test is, therefore, an excellent supplement to the Mitscherlich test. But, although the former is very delicate, I would not recommend it as a substitute for the Mitscherlich test. Selmi states that animal material like brain, which contains organic phosphorus compounds, yields after putrefaction a distillate that often gives a black precipitate with silver nitrate solution. This will impart a green tinge in the Blondlot-Dussard test. And, moreover, it is difficult to get zinc absolutely free from phosphorus.

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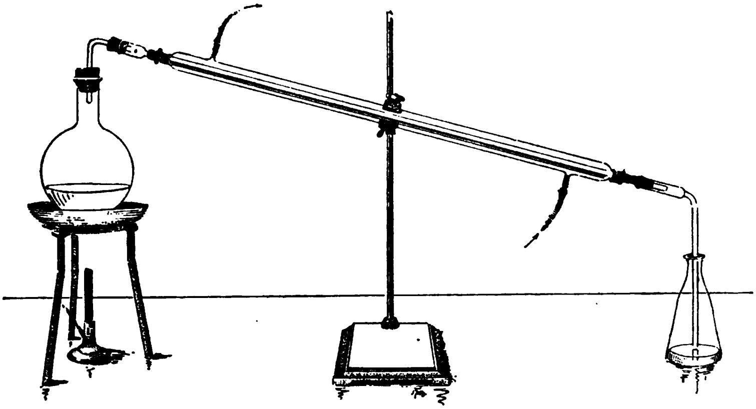
[1] Hilger and Nattermann, *Forschungsberichte über Lebensmittel und ihre Beziehungen zur Hygiene,* etc., 4, 255 (1897).

Metabolism in Phosphorus Poisoning.—Thus far no satisfactory explanation for all the changes that occur during phosphorus poisoning has been given. Metabolism in human beings and animals during phosphorus poisoning has been studied experimentally. The results of these experiments show diminution both in carbon dioxide produced and in oxygen consumed. The quantity of proteïn matter broken down is considerably in excess of the normal. This decomposition is responsible for a marked increase in elimination of nitrogenous compounds. The total elimination of nitrogen is enormous, amounting even to three times that ordinarily excreted. Most of the nitrogen passes off as urea. Usually the quantity of ammonia in the urine is increased. The urine of persons poisoned by phosphorus nearly always contains the amido-acids, leucine and tyrosine, as well as sarcolactic acid. The urine remains acid to the end. The quantity of phosphoric and sulphuric acids is also increased.

Further Examination of Distillate.—When phosphorescence has been distinctly observed in the Mitscherlich apparatus, it is advisable to stop distillation and change the Liebig condenser to its customary inclined position. This simpler method of distilling is shown in Fig. 5 and should always be used in toxicological analysis when there is no occasion to test for phosphorus.

Since the several poisons of Group I are not equally volatile, it is best to collect the distillate in two or three fractions. The first will contain most of the easily volatile substances like hydrocyanic acid, chloroform, ethyl alcohol, acetone, iodoform and nitrobenzene. The others (second and third) will contain substances less easily volatile with steam like carbolic acid, aniline, chloral hydrate and carbon disulphide. This must not be understood to mean that the first part of the distillate will be free from substances that volatilize with difficulty, and the latter part free from those that volatilize easily. In the main such will be the separation, but either part of the distillate may contain traces of substances which will appear in larger quantity in the other part.

FIG. 5.



Simple Distillation with Liebig Condenser.

The proper procedure is to distil until 5 to 8 c.c. of liquid have been collected. Divide the distillate into several portions and test for hydrocyanic acid, chloroform, ethyl alcohol, acetone, and, if necessary, also for iodoform and nitrobenzene. Use the second and third portions (10 to 15 c.c.) to test for carbolic acid, aniline, chloral hydrate and carbon disulphide.

Several of these volatile substances have a characteristic odor, which makes it possible to recognize them with great certainty in the original material and especially in the distillate. First, test the distillate for each individual substance by its most characteristic reaction. Test for hydrocyanic acid by the Prussian blue or sulphocyanate reaction; for ethyl alcohol, acetone and acetaldehyde by Lieben's reaction; for carbolic acid and aniline by Millon's reaction; for chloroform, chloral hydrate and iodoform by the phenylisocyanide reaction; for aniline with calcium hypochlorite solution; and finally, for carbon disulphide with lead acetate and potassium hydroxide solutions.

When there is reason to believe that a certain substance is present, confirm the result by making other characteristic tests. It is seldom necessary to examine the distillate for all the members of the group.

Hydrocyanic Acid.

When the material contains an appreciable quantity of free hydrocyanic acid (HCN), it can be detected by its characteristic odor which is also apparent in the distillate.

In poisoning by hydrocyanic acid or potassium cyanide, the breath of the person frequently has the odor of this acid. This is also noticeable at the autopsy, provided it be made soon after death. The parts of the body to be removed for chemical examination are stomach and contents, blood and some of the organs rich in blood like liver, brain and heart and in certain cases also the urine. In every instance the organs should be examined for hydrocyanic acid as soon as they have been removed. This is necessary, because hydrocyanic acid is decomposed with great ease. It is usually impossible on this account to detect hydrocyanic acid in organs that have begun to putrefy. Cases of poisoning by potassium cyanide are on record, where hydrocyanic acid could not be found even a few hours after death. On the other hand, it has been detected even eight days after death.

Our knowledge of the fate of hydrocyanic acid in the living organism is unsatisfactory. Since carbon dioxide of the blood liberates hydrocyanic acid from its salts, at least to some extent, it may be present in the blood partly combined with sodium and potassium and partly as the free acid. Consequently, many authors suppose that essentially all hydrocyanic acid taken into the system is eliminated by the lungs. Kobert (Lehrbuch der Intoxikationen) states that he has found hydrocyanic acid in the urine after poisoning by potassium cyanide. Inasmuch as free hydrocyanic acid is always found after poisoning by this substance, when organs have not begun to putrefy, it is generally supposed that hydrocyanic acid is not completely decomposed in animal metabolism.

Schönbein's Test for Hydrocyanic Acid.—It is best to make a special test for hydrocyanic acid before distilling. The principle of this test is that the vapor of hydrocyanic acid immediately produces a deep blue color upon a strip of paper[1] moistened with tincture of guaiac and copper sulphate solution. Acidify a portion of the original material in a small flask with tartaric acid solution. Then suspend in the flask a strip of "guaiac-copper" paper without letting it touch the liquid. Gently warm the contents of the flask upon the water-bath. Hydrocyanic acid is not present, unless the paper be turned blue. But the only conclusion to be drawn from a positive test is that hydrocyanic acid may be present, because other substances like ammonia, volatile ammonium compounds and especially oxidizing agents like ozone, hydrogen dioxide, nitric acid and chlorine will turn the paper blue. Consequently, Schönbein's test for hydrocyanic acid, although very delicate, cannot alone be accepted as conclusive. The test has nothing to do directly with hydrocyanic acid but depends upon the ozonizing action of its vapor.

When a distillate is to be tested, instead of the original material, put a small portion in a porcelain dish and add a drop of freshly prepared guaiac tincture and then a drop of copper sulphate solution. A blue color indicates hydrocyanic acid.

The tests to be described, if positive, afford unquestionable evidence of the presence of hydrocyanic acid. This acid distils easily with steam and most of it will be in the first 5 to 10 c.c. of distillate. Therefore, in a fractional distillation, the first fraction should be examined for hydrocyanic acid.

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[1] Prepare "guaiac-copper" paper by saturating strips of filter-paper with freshly prepared, 10 per cent. alcoholic tincture of resin of guaiac. Dry these strips in air and moisten before using with very dilute, aqueous copper sulphate solution (1 : 1000).

1. **Prussian Blue Test.**—Add to the solution (distillate) a small quantity of potassium hydroxide solution; then 1 or 2 drops of ferrous sulphate solution and 1 drop of ferric chloride solution. Shake well and warm gently. Finally acidify with dilute hydrochloric acid. If much hydrocyanic acid be present, a precipitate of Prussian blue will appear immediately. But if the quantity be small, the solution will have merely a blue or bluish-green color. After a long time (10 to 12 hours) a flocculent precipitate of Prussian blue will settle to the bottom of the test-tube. The limit of delicacy is 1 : 5,000,000.[1] The reactions[2] are:

1. $FeSO_4 + 2KOH = Fe(OH)_2 + K_2SO_4$,
2. $Fe(OH)_2 + 2KCN = Fe(CN)_2 + 2KOH$,
3. $Fe(CN)_2 + 4KCN = K_4Fe(CN)_6$,
4. $4FeCl_3 + 3K_4Fe(CN)_6 = Fe_4(Fe(CN)_6)_3 + 12KCl$.

2. **Sulphocyanate Test.**—Add to a portion of the distillate 3 or 4 drops of potassium hydroxide solution and then a small quantity of yellow ammonium sulphide solution. Evaporate to dryness upon the water-bath. Dissolve the residue in a small quantity of water, and acidify with dilute hydrochloric acid. Filter through double paper to remove sulphur, and add to the filtrate a drop of ferric chloride solution. If the distillate contained hydrocyanic acid, a reddish to blood-red color will appear. This is due to ferric sulphocyanate ($Fe(SCN)_3$), or to a double sulphocyanate of iron and potassium ($Fe(SCN)_3.3KSCN$ or $Fe(SCN)_3.9KSCN$). The limit of delicacy is 1 : 4,000,000. The reactions are:

1. $KCN + (H_4N)_2S_x = KSCN + (H_4N)_2S_{x-1}$,
2. $6KSCN + FeCl_3 = Fe(SCN)_3.3KSCN + 3KCl$.

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[1] Link and Moeckel, *Zeitschrift für analytische Chemie,* 17, 455 (1878).

[2] $Fe(OH)_3$, at first precipitated, is dissolved by HCl and forms $FeCl_3$.

3. **Vortmann's[1] Nitroprusside Test.**—Add to a portion of the distillate a few drops of potassium nitrite solution; then 2 to 4 drops of ferric chloride solution and enough dilute sulphuric acid to give a bright yellow color. Heat to boiling, add sufficient ammonium hydroxide solution to remove excess of iron and filter. Add to the filtrate 1 or 2 drops of very dilute ammonium sulphide solution. If the solution contained hydrocyanic acid, a violet color will appear and pass through blue, green and yellow. The limit of delicacy is 1 : 312,000. This is the reverse of the nitroprusside test for hydrogen sulphide and is due to conversion of hydrocyanic acid to $K_2Fe(NO)(CN)_5$, potassium nitroprusside.

4. **Silver Nitrate Test.**—Acidify a portion of distillate with dilute nitric acid, and add silver nitrate solution. If hydrocyanic acid be present, a white, curdy precipitate of silver cyanide will appear. Excess of ammonium hydroxide solution will readily dissolve this precipitate. The limit of delicacy is 1 : 250,000.

When a dilute aqueous solution of hydrochloric acid is distilled, the acid does not pass into the distillate. The precipitate, therefore, caused by silver nitrate solution cannot possibly be silver chloride, because at first nothing but pure water distils from a 1 per cent. or weaker solution of hydrochloric acid. To remove hydrochloric acid, if present, redistil the first distillate over borax. This will retain hydrochloric but not hydrocyanic acid. It is advisable to collect upon a filter and dry the precipitate caused by silver nitrate solution. If silver cyanide be heated in a bulb-tube, it will form metallic silver and cyanogen gas. The latter may be recognized by its characteristic odor.[2] The reaction is:

$$2AgCN = 2Ag + (CN)_2.$$

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[1] *Monatshefte für Chemie*, 7, 416 (1886).

[2] Owing to the very poisonous character of cyanogen gas, it is safer to ignite the gas at the mouth of the bulb-tube. Cyanogen gas burns with a purple-colored flame. Tr.

5. Picric Acid Test.—Make alkaline a portion of distillate with potassium hydroxide solution and heat gently (50 to 60°) with a few drops of picric acid solution. If hydrocyanic acid be present, the solution will be colored blood-red. This is due to formation of potassium isopurpurate. This test is not as delicate, nor as characteristic of hydrocyanic acid as the other tests. If hydrogen sulphide be present, as it frequently is in distillates from animal matter, picric acid solution will produce a red color owing to formation of picraminic acid.

Quantitative Determination of Hydrocyanic Acid.—To determine hydrocyanic acid quantitatively, acidify a weighed portion of material with dilute sulphuric or tartaric acid and distil. Determine the quantity of hydrocyanic acid in the distillate either gravimetrically or volumetrically. If the former method be used, collect the precipitate of silver cyanide upon a filter and dry at 100°; or ignite the precipitate in a weighed porcelain crucible, and determine the quantity of metallic silver obtained. If hydrochloric acid be present in the distillate, redistil once over borax. The distillate will then be free from hydrochloric acid.

Detection of Hydrocyanic Acid in Presence of Potassium Ferrocyanide.—When material contains non-poisonous potassium ferrocyanide, hydrocyanic acid will appear in the distillate from a solution acidified with tartaric acid. In an experiment, where 1 per cent. potassium ferrocyanide solution was distilled with 0.03 grams of tartaric acid, the distillate contained considerable hydrocyanic acid. Carbon dioxide, passed into hot, aqueous potassium ferrocyanide solution, will liberate hydrocyanic acid even at water-bath temperature (75°). To test for potassium ferrocyanide beforehand, shake some of the original material with water and filter. Test the filtrate with ferric chloride solution and dilute hydrochloric acid. If there be a precipitate of Prussian blue, potassium ferrocyanide is present. To detect free hydrocyanic acid, potassium or sodium cyanide[1] with certainty, in presence of potassium ferrocyanide, add to the material acid sodium carbonate in not too small quantity and distil. Even long distillation over free flame by this method will liberate hydrocyanic acid only from simple cyanides and not from potassium ferrocyanide.

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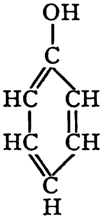
[1] Mercuric cyanide is an exception.

Detection of Mercuric Cyanide.—When an aqueous solution of mercuric cyanide, which is exceedingly poisonous, is distilled with tartaric acid, the distillate will contain hydrocyanic acid only when a large quantity of mercuric cyanide is present. Distillation of 100 c.c. of 1 per cent. aqueous mercuric cyanide solution yields a distillate which gives the Prussian blue test distinctly. But, if the quantity of mercuric cyanide be less and the solution very dilute (for example, 100 c.c. of 0.01 per cent. solution), there will be no trace of hydrocyanic acid in the distillate, even though the solution be strongly acidified with tartaric acid. If, however, a few c.c. of freshly prepared hydrogen sulphide water be added and distillation be resumed, mercuric cyanide will be completely decomposed and the distillate will contain hydrocyanic acid.

Detection of Mercuric Cyanide in Presence of Potassium Ferrocyanide.—The method for detecting hydrocyanic acid from simple cyanides, in presence of potassium ferrocyanide, is not applicable to mercuric cyanide. Long distillation, even from saturated acid sodium carbonate solution, gives no trace of hydrocyanic acid. But distillation in presence of not too small a quantity of acid sodium carbonate, after addition of a few c.c. of freshly prepared, saturated hydrogen sulphide solution, liberates hydrocyanic acid from mercuric cyanide and not from potassium ferrocyanide. It is possible to detect hydrocyanic acid by this method, when a very small quantity of mercuric cyanide is mixed with considerable potassium ferrocyanide. For example, 0.01 grams of mercuric cyanide in 100 c.c. of 10 per cent. potassium ferrocyanide solution can be detected. If potassium ferrocyanide be distilled directly with hydrogen sulphide without addition of acid sodium carbonate, the distillate will contain considerable hydrocyanic acid.

CARBOLIC ACID.



Carbolic acid distils quite easily with steam. Yet, to remove the last traces, long-continued distillation is necessary. If fractional distillation be made, when carbolic acid is present, this substance will appear in the first and second fractions and even in the third. Usually carbolic acid can be recognized by its peculiar odor. When much carbolic acid is present, the distillate is milky. Colorless or reddish globules may be seen floating in the liquid. Excess of potassium or sodium hydroxide solution will dissolve carbolic acid and render the distillate perfectly clear. Pure, anhydrous carbolic acid melts at 40 to 42° and distils at 178 to 182°.

Decomposition of proteïn substances produces phenol and especially para-cresol in small quantity. Traces of phenols can almost always be detected in distillates from animal matter in an advanced stage of decomposition. Millon's reagent, and usually bromine water, will give positive tests with such distillates.

Fate of Carbolic Acid in the Animal Body.—The human body very rapidly absorbs carbolic acid, of which the greater part is converted into phenyl-sulphuric acid ($(C_6H_5O).SO_2.OH$). When the quantity of carbolic acid is very large, a portion is also converted into phenyl-glycuronic acid. The non-toxic, alkali salts of these conjugate acids are then eliminated in the urine. Considerable carbolic acid is oxidized within the body to dihydroxy-benzenes, namely pyrocatechol ($C_6H_4(OH)_2(1,2)$) and hydroquinol ($C_6H_4(OH)_2(1,4)$). These enter into synthesis with sulphuric acid and appear in urine as ethereal salts of sulphuric acid. The dark color of "carbolic urine" is largely due to further oxidation of hydroquinol, whereby colored products (quinone ?) are formed. In carbolic acid poisoning, urine often has a pronounced dark color (greenish to black). Urine in other cases is amber-yellow at first, but standing in air gives it a deeper color. When carbolic acid poisoning is suspected, the urine should be examined chemically. "Carbolic urine" differs from normal, human urine in being nearly free from sulphuric acid,[1] the so-called "preformed sulphuric acid." Consequently barium chloride solution, in presence of excess of acetic acid, gives only a slight precipitate of barium sulphate or none at all. Filter, when there is a precipitate, and warm the clear filtrate with a few c.c. of concentrated hydrochloric acid. An abundant precipitate of barium sulphate will usually appear. The mineral acid decomposes phenyl-sulphuric acid into phenol and sulphuric acid. Normal, human urine contains considerably more "sulphuric acid" (A—sulphuric acid) than "ethereal sulphuric acid" (B—sulphuric acid). The average proportion between the two being: A — SO_4 : B —

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[1] This is sulphuric acid present in urine as sulphates. It is also termed "preformed sulphuric acid," by which is meant that it enters the body as such. In this respect it differs from "ethereal," or "conjugate" sulphuric acids, which result from syntheses within the body.

$SO_4 = 10:1$. Barium chloride solution, added to normal urine in presence of acetic acid, produces a heavy precipitate of barium sulphate.

Distribution of Carbolic Acid After Poisoning.—C. Bischoff[1] examined organs, removed from a man who died 15 minutes after taking 15 c.c. of liquid carbolic acid, and found the poison distributed, as stated in the table below. The organs in this case were perfectly fresh. Only a small portion of the stomach was received.

Weight.		Organ.	Phenol.	
242	grams	Contents of stomach and intestine	0.171	grams.
112	"	Blood	0.028	"
1480	"	Liver	0.637	"
322	"	Kidney	0.201	"
1445	"	Brain	0.314	"

Bischoff distilled with steam until the distillate gave no further precipitate with bromine water. The results show how rapidly carbolic acid is absorbed, and how soon it is distributed throughout the body.

E. Baumann[2] has published certain facts relating to the quantity of carbolic acid formed during putrefaction of proteïn substances. Baumann states that he obtained from 100 grams of fresh pancreas and 100 grams of moist fibrin, mixed with 250 c.c. of water, after 6 days of putrefaction 0.073 to 0.078 grams of tribromophenol, corresponding to 0.0208 to 0.022 grams of phenol.

Urine gives a distinct test for carbolic acid 15 minutes after the poison has been taken by the mouth, or hypodermically. This shows how rapidly carbolic acid is absorbed. Most of the carbolic acid absorbed is eliminated in 4 or 5 hours. Schaffer[3] found the quantity of conjugate sulphuric acid in urine to increase in exact proportion to the quantity of carbolic acid taken.

Carbolic acid may be recognized by the following tests:

1. **Millon's (Plugge's) Test.**—Millon's reagent, heated with a solution containing only a trace of carbolic acid, produces a red to dark-red color. This is a very delicate test and shows phenol even in extremely dilute solutions. A distinct red color is given by 0.00005 grams of carbolic acid diluted 1:100,000. But this test is not characteristic of

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[1] *Berichte der Deutschen chemischen Gesellschaft,* 16, 1337 (1883).

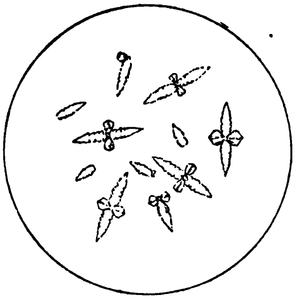
[2] *Berichte der Deutschen chemischen Gesellschaft,* 10, 685 (1877) and *Zeitschrift für physiologische Chemie,* 1, 61 (1877–78).

[3] *Journal für praktische Chemie,* Neue Folge 18, 282 (1878).

carbolic acid, because several other aromatic compounds behave similarly. This is true of derivatives of mon-acid phenols like the three cresols, salicylic acid, para-hydroxybenzoic acid, tyrosine, hydro-para-cumaric acid and para-hydroxy-phenyl-acetic acid.[1] Aniline heated with Millon's reagent also gives a dark-red color.

2. **Bromine Water Test.**—This reagent produces a yellowish-white, crystalline precipitate, even with very dilute carbolic acid solutions. This precipitate is a mixture of considerable tribromophenol ($C_6H_2.Br_3.OH$) with a small quantity of tribromophenol bromide ($C_6H_2.Br_3.OBr$). It usually consists of radiating clusters of shining, needle-like crystals (Fig. 6). It is a very delicate test for carbolic acid. Phenol diluted 1 : 50,000 yields, after some time, a precipitate made up in part of well-formed crystals.

FIG. 6.



Tribromophenol Crystals.

3. **Ferric Chloride Test.**—Diluted ferric chloride solution imparts a blue-violet color to aqueous carbolic acid solutions. Addition of dilute hydrochloric or sulphuric acid changes this color to yellow. This test is not as delicate as 1 and 2.

4. **Calcium Hypochlorite Test.**—Add a few c.c. of ammonium hydroxide solution to a dilute, aqueous carbolic acid solution, and then 2 or 3 drops of freshly prepared calcium hypochlorite solution. Gentle warming will pro-

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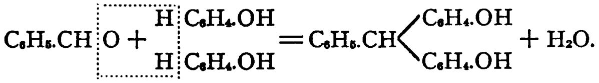
[1] The last two acids are also formed in the putrefaction of protein substances, but they are not volatile with steam. Salicylic acid, however, passes into the distillate, at least in sufficient quantity for detection with Millon's reagent.

duce a blue color. Very dilute carbolic acid solutions give only a green to blue-green color.

5. **H. Melzer's Benzaldehyde Test.**[1]—Concentrated sulphuric acid produces a dark brown color with benzaldehyde. Addition of carbolic acid and heat change this color to red. There is formed at the same time a resinous precipitate, insoluble in water and acids, but soluble in alkalies with a splendid, violet-blue color. The final product is di-para-dihydroxy-triphenyl-methane, the reaction for its formation being:



$$C_6H_5.CH\boxed{O + \begin{matrix}H\\H\end{matrix}}\begin{matrix}C_6H_4.OH\\C_6H_4.OH\end{matrix} = C_6H_5.CH\begin{matrix}\diagup C_6H_4.OH\\ \diagdown C_6H_4.OH\end{matrix} + H_2O.$$

Procedure.—Add 2 c.c. of pure, concentrated sulphuric acid to 1 c.c. of the solution (distillate) to be tested for carbolic acid, then 1 or 2 drops of benzaldehyde and heat. The mixture, at first yellowish-brown, will become dark-red. At the same time a red, resinous substance will appear, unless the solution be too dilute. When cold, add 10 c.c. of water and enough potassium hydroxide solution to give a distinct alkaline reaction. If carbolic acid be present, a violet-blue color will appear. To obtain this coloring-matter, acidify the solution, extract with ether and evaporate the solvent. Alkalies, added to alcoholic solutions of the coloring-matter, produce a blue color which acids discharge. This is a delicate test. One drop of benzaldehyde, added to 1 c.c. of 0.05 per cent. carbolic acid solution (=0.0005 grams of carbolic acid), will still give the blue color very distinctly.

Quantitative Determination of Carbolic Acid.—Either of two volumetric methods may be used to determine carbolic acid quantitatively.

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[1] *Zeitschrift für analytische Chemie*, 37, 345 (1898).

1. Koppeschaar-Beckurts[1] Method.

The following standard solutions are required:

1. N/100 Potassium bromide solution, containing 5.939 grams of KBr per 1000 c.c.
2. N/100 Potassium bromate solution, containing 1.667 grams of $KBrO_3$ per 1000 c.c.
3. N/10 Sodium hyposulphite solution, containing 24.83 grams of $Na_2S_2O_3.5H_2O$ per 1000 c.c.
4. Potassium iodide solution, containing 125 grams of KI per 1000 c.c.

Procedure.—Put about 25 c.c. of 0.1 per cent. carbolic acid solution (distillate) into a flask having a tight, glass stopper. Add 50 c.c. each, of standard potassium bromide and potassium bromate solutions, then 5 c.c. of pure, concentrated sulphuric acid and shake vigorously. The gradually increasing opalescence of the solution becomes more and more marked, as tribromophenol and tribromophenol bromide are precipitated. The yellow color which soon appears shows excess of bromine. Open the flask in 15 minutes, add 10 c.c. of potassium iodide solution and titrate free iodine in 5 minutes with N/10 sodium hyposulphite solution. Excess of free bromine, and also precipitated tribromophenol bromide, act upon potassium iodide, the latter reaction being:

$$C_6H_2.Br_3.OBr + KI = I + C_6H_2.Br_3.OK.$$

Each molecule of carbolic acid in the Koppeschaar-Beckurts method of titration requires 6 atoms of bromine which in turn set 6 atoms of iodine free.

Calculation.—Sulphuric acid liberates 0.1994 grams of bromine from 50 c.c. of N/100 potassium bromide solution, and 0.0399 grams from 50 c.c. of N/100 potassium bromate solution, or 0.2393 grams in all. Since each molecule of carbolic acid requires 6 atoms of bromine, the following proportion shows how much carbolic acid can be converted into tribromophenol by 0.2393 grams of bromine:

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[1] *Archiv der Pharmacie*, 24, 570 (1886).

$$\begin{array}{ccccccc} 6Br & & C_6H_5.OH & & & & \\ 480 & : & 94 & = & 0.2393 & : & x, \end{array}$$

and x = 0.0469 grams.

1 c.c. of N/10 sodium hyposulphite solution corresponds to 0.008 grams of bromine which can convert 0.00156 grams of carbolic acid into tribromophenol, for

$$480 : 94 = 0.008 : x,$$

and x = 0.00156 grams. Therefore, for every c.c. of N/10 sodium hyposulphite solution, used to combine with iodine set free by bromine, subtract 0.00156 grams from 0.0469 grams of carbolic acid. This determines the quantity of carbolic acid in the measured volume of solution.

2. Kossler-Penny[1] Method.

In this method carbolic acid in alkaline solution is converted by iodine into tri-iodophenol ($C_6H_2.I_3.OH$), the reactions being:

1. $6NaOH + 3I_2 = 3NaOI + 3NaI.$
2. $C_6H_5.OH + 3NaOI = 3NaOH + C_6H_2.I_3.OH.$

In the cold, this reaction takes place quite slowly, but it is hastened by warming to 60°.

Procedure.—Add a measured quantity of N/10 potassium hydroxide solution to an aqueous carbolic acid solution (distillate), until the reaction is strongly alkaline. Warm gently by immersing the vessel in water heated to 60°, and add 10 to 15 c.c. more of N/10 iodine solution than has been used of N/10 potassium hydroxide solution. Cool, acidify the solution with dilute hydrochloric acid and titrate free iodine with N/10 sodium hyposulphite solution.

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[1] Hoppe-Seyler's *Zeitschrift für physiologische Chemie,* 17, 117 (1892).

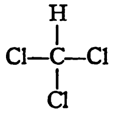
Calculation.—The reaction above shows that each molecule of carbolic acid requires 6 atoms of iodine, or 127 parts of iodine by weight (= 1 atom) correspond to $C_6H_5.OH/6 = 94/6 = 15.67$ parts of carbolic acid by weight. Therefore, 1 c.c. of N/10 iodine solution, containing 0.0127 grams of iodine, and also 1 c.c. of N/10 sodium hyposulphite solution are equivalent to 0.001567 grams of carbolic acid.

In determining carbolic acid in urine, the regular occurrence of phenols must not be overlooked. After a mixed diet, the quantity of normal human urine passed in 24 hours will yield approximately 0.03 grams of phenols. The substances present are phenol, but more especially para-cresol.

In certain diseases where there is excessive bacterial decomposition within the organism, in the intestines for example, urine contains more of these phenols and, consequently, more conjugate sulphuric acids. Even external application of carbolic acid, for instance the use of carbolic acid water as a lotion, is sufficient to increase the quantity of phenyl-sulphuric acid in urine.

Detection of Carbolic Acid in Presence of Aniline.—Aniline closely resembles carbolic acid in behavior towards Millon's reagent and bromine water. But the two substances can be easily separated. Add potassium hydroxide solution in large excess to the solution and distil. The distillate will contain aniline alone. Or make the solution strongly acid with dilute sulphuric acid, and extract with ether which will dissolve only carbolic acid. Evaporate the ether extract at a moderate temperature and examine the residue.

CHLOROFORM.



$$Cl-\overset{\displaystyle H}{\underset{\displaystyle Cl}{C}}-Cl$$

Chloroform distils easily with steam and appears in the first fraction in largest quantity. For example, the first 15 c.c. of distillate, from a mixture of 5 drops of chloroform and 200 c.c. of water acidified with tartaric acid, will contain nearly all the chloroform. When much chloroform is present, it will separate from the distillate as heavy, colorless globules, whereas a small quantity will remain in solution. This solution usually has the characteristic odor and sweetish taste of chloroform. Pure chloroform boils at 62°.

Chloroform may be recognized by the following tests:

1. **Phenylisocyanide Test.**—Add 1 or 2 drops of aniline to the chloroform solution (distillate), and then a few c.c. of aqueous, or alcoholic potassium hydroxide solution. Gentle application of heat will produce phenylisocyanide. The penetrating and very repulsive odor of this compound is easily recognized.

$$CHCl_3 + C_6H_5.NH_2 + 3KOH = C_6H_5.NC + 3KCl + 3H_2O.$$

This is a very delicate test for chloroform. A. W. Hofmann states that it will show with great certainty 1 part of chloroform in 5000 to 6000 parts of alcohol. Chloral, chloral hydrate, bromoform, iodoform and tetrachloromethane also give this test. The fact that aniline boiled with potassium hydroxide solution gives a peculiar, faintly ammoniacal odor, even when chloroform is absent, must not be overlooked. There is small chance, however, of confusing this odor with the repulsive smell of phenylisocyanide. In doubtful cases, warm some water, containing a drop of aniline and a trace of chloroform (one-fourth of a drop or less), with potassium hydroxide solution and compare the odor with that in question.

2. **Fehling's Solution Test.**—Warm an aqueous chloroform solution (distillate) with Fehling's solution. A red precipitate of cuprous oxide will appear.

3. **Silver Reduction Test.**—Add excess of ammonium hydroxide solution to silver nitrate solution and then some of the chloroform solution. Heat will produce a black precipitate of metallic silver. Tests 2 and 3 are due to formic

acid, resulting from decomposition of chloroform by the alkali.

$$CHCl_3 + 4KOH = 3KCl + H.COOK + 2H_2O.$$

These reactions are not characteristic of chloroform, because many reducing substances, other than formic acid, behave similarly. The aldehydes, for instance, reduce Fehling's and ammoniacal silver nitrate solutions.

4. **Resorcinol Test.**—Dissolve about 0.1 grams of resorcinol in 1 or 2 c.c. of water, and add a few drops of sodium hydroxide solution. Then add the solution to be tested and boil the mixture. A yellowish-red to red color, attended by brilliant fluorescence even in very dilute solution, will appear. Chloral, bromal, bromoform and iodoform also give this test.

5. **Lustgarten's[1] Naphthol Test.**—Dissolve about 0.05 grams of α- or β-naphthol in 1 or 2 c.c. of 33 per cent. aqueous potassium hydroxide solution. Warm to 50° and add the solution to be tested. Chloroform will produce an evanescent, blue color which, in contact with air, will change to green and then to brown. Acidification of the blue solution will precipitate naphthol colored by a red dye-stuff. This precipitate is usually brick-red. Chloral, bromal and bromoform also give this test.

Ludwig's Quantitative Determination of Chloroform in the Cadaver (B. Fischer[2]).—Mix a weighed portion of material with water, and distil as long as there is any chloroform. To tell when this point is reached, apply the phenylisocyanide test to a few c.c. of liquid collected at the end of distillation. Add some calcium carbonate to combine with free hydrochloric acid. Warm the distillate to about 60° and draw washed air through it by suction. Pass this air

[1] *Monatshefte für Chemie*, 3, 715 (1882).

[2] *Jahresbericht des chemischen Untersuchungsamtes der Stadt Breslau* für die Zeit vom 1. April, 1894, bis 31. März, 1895.

through a combustion-tube, heated to high temperature, and then into silver nitrate solution acidified with nitric acid. Weigh the precipitated AgCl(N), and calculate the percentage of chloroform on the basis of the proportion:

$$3AgCl : CHCl_3 = N : x.$$

Chloroform, heated with steam above 200°, is decomposed into carbon monoxide, hydrochloric and formic acids.

$$1.\ CHCl_3 + H_2O = CO + 3HCl.$$
$$2.\ CHCl_3 + 2H_2O = H.COOH + 3HCl.$$

In a series of experiments B. Fischer has shown that the stomach, stomach contents and blood, of a person who has not taken chloroform, give no volatile chlorine compounds under these conditions. By this method B. Fischer found in the cadaver of a laborer, who had died during chloroform narcosis, the following quantities of chloroform:

Weight.	Organ.	Chloroform.
985 grams	Stomach and contents and parts of the intestine	0.1 grams.
780 "	Lungs and blood from the heart	0.055 "
445 "	Portions of spleen, kidneys and liver	traces
480 "	Brain	0.07 "

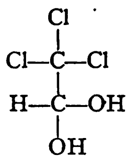
From these results it appears that most of the chloroform was in the brain and blood.

6. Conversion of Chloroform into Hydrocyanic Acid.—Put a portion of distillate into a pressure-flask[1] with some solid ammonium chloride and 2 or 3 c.c. of alcoholic potassium hydroxide solution. Seal the flask and heat to boiling (0.5 to 1 hour) in a water-bath. When the solution is cold, test for hydrocyanic acid by the Prussian blue reaction. The reaction by which hydrocyanic acid is formed from chloroform is:

$$CHCl_3 + H_3N + 3KOH = HCN + 3KCl + 3H_2O.$$

[1] An ordinary citrate of magnesium bottle is a convenient apparatus for this test. Wrap a towel around the bottle, place it in the water-bath and gradually raise the temperature to boiling. Do not remove the bottle until it is cold. Tr.

CHLORAL HYDRATE.



Chloral hydrate distils with steam from a solution acidified with tartaric acid, and appears in the distillate as such. Complete distillation of a large quantity of chloral hydrate requires considerable time. The distillate gives the chloroform tests, but does not have the characteristic odor of this substance. The odor of chloroform appears, however, when the distillate is warmed with potassium hydroxide solution, and, if the quantity of chloral hydrate be large, the liquid becomes milky owing to separation of finely divided chloroform.

Test the distillate further, by heating a portion for 30 minutes under a reflux condenser with calcined magnesium oxide (MgO). Formic acid is produced by decomposition of chloral hydrate.

$$2CCl_3.CH(OH)_2 + MgO = 2CHCl_3 + Mg(OOCH)_2 + H_2O.$$

Filter to remove undissolved magnesium oxide, and concentrate the filtrate by evaporation upon the water-bath. Make two tests for formic acid with this solution.

1. Warm one portion with a few drops of mercuric chloride solution. Formic acid, if present, will precipitate white, mercurous chloride (Hg_2Cl_2).

$$Mg(OOCH)_2 + 4HgCl_2 = 2Hg_2Cl_2 + MgCl_2 + 2HCl + 2CO_2.$$

2. Warm the second portion with silver nitrate solution. Formic acid will cause deposition of metallic silver.

$$Mg(OOCH)_2 + 4AgNO_3 = 4Ag + Mg(NO_3)_2 + 2HNO_3 + 2CO_2.$$

Jaworowski[1] suggests the following tests for chloral hydrate:

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[1] *Pharmaceutische Zeitung für Russland,* 33, 373, and *Zeitschrift für analytische Chemie,* 37, 60 (1898).

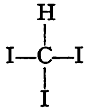
1. Add a few drops of Nessler's reagent to an aqueous chloral hydrate solution. It will produce a brick-red precipitate, the color of which will change after a while to a dirty yellowish-green (Hg_2I_2).

2. Boil a few c.c. of chloral hydrate solution with a small quantity of sodium hyposulphite solution. This will give a turbid liquid with a brick-red color. A few drops of potassium hydroxide solution will remove the turbidity and change the color to brownish-red.

Fate of Chloral Hydrate in the Human Body.—A very small quantity of chloral hydrate, when administered by the mouth, passes into the urine unchanged. The greater part is converted into a conjugate glycuronic acid, called urochloralic acid (constitution ?). This substance, heated with dilute sulphuric acid, is decomposed with addition of water into trichlorethyl alcohol ($CCl_3.CH_2.OH$) and glycuronic acid ($HO.CO.(CH.OH)_4.CHO$).

Silver solution and alkaline solutions of copper and bismuth are reduced, when heated with urochloralic acid. The acid and its salts are laevo-rotatory. Hence "chloral urine" resembles "sugar urine" in many respects, but it is laevo-rotatory, whereas the latter is dextro-rotatory.

IODOFORM.



$$\begin{array}{c} H \\ | \\ I—C—I \\ | \\ I \end{array}$$

Iodoform distils quite easily with steam and gives a milky distillate with a characteristic saffron-like odor. Extract this distillate with ether, and carefully test the residue left by evaporating the solvent. If much iodoform be present, it will form yellow or merely yellowish hexagonal plates. Dissolve the ether residue in a small quantity of alcohol, and use this solution in the following tests:

1. **Lustgarten's[1] Test.**—Gently warm a few drops of alcoholic iodoform solution in a test-tube with a small quantity of sodium phenolate ($C_6H_5.ONa$) solution. If iodoform be present, a red substance (rosolic acid?) will be

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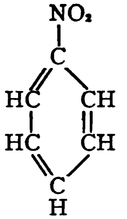
[1] *Monatshefte für Chemie,* 3, 715 (1882).

deposited at the bottom of the tube. A few drops of dilute alcohol will dissolve this precipitate with a carmine-red color. Prepare sodium phenolate solution by mixing 20 grams of phenol with 40 grams of sodium hydroxide and 70 grams of water. Resorcinol may be substituted for phenol without in the least affecting the delicacy of the test. But the isomeric dihydroxybenzenes, pyrocatechol and hydroquinol, cannot be used.

2. **Hofmann's Phenylisocyanide Test.**—Heat alcoholic iodoform solution with alcoholic potassium hydroxide solution and a drop of aniline. Iodoform like chloroform, if present, will produce the phenylisocyanide odor.

NITROBENZENE.



Nitrobenzene distils quite easily with steam, and appears in the distillate as yellowish globules. These are heavier than water and have a characteristic odor.

Nitrobenzene[1] has a very strong poisonous action. Administration of very small quantities of this compound has produced death in human beings. There are records in the literature of several cases in which 20 drops, and even 7 to 8 drops, have caused fatal results. But, on the other hand, complete recovery has followed poisoning by much larger doses. Fatal poisonings have come even from inhaling nitrobenzene vapor. Within recent years nitrobenzene has been used for suicidal purposes. Women have used it as an abortifacient.

Nitrobenzene changes the appearance of the blood. It gives it a chocolate color, and renders it incapable of uniting with oxygen. The blood of living animals poisoned by nitrobenzene has been found to contain only 3 to 5 per cent. of oxygen by volume, as against 17 per cent., the normal quantity. "Nitrobenzene blood" has a very dark color, which it retains even after agitation with air. Spectroscopic examination of such blood has shown an absorption-band, lying between C and D, associated with the oxyhaemoglobin band. A part of the nitrobenzene passes as such into the urine, whereas another part appears to be changed in metabolism. (See Weissenstein, Inaugural-Dissertation, Würzburg, 1892.)

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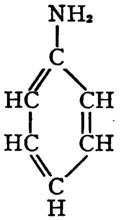
[1] Para-chloro-nitro-benzene ($C_6H_4.NO_2.Cl(1,4)$), which acts as a vigorous poison, has been used of late as the starting-point in preparing phenacetine. Cases of severe poisoning from this substance have occurred in several chemical factories. Para-chloro-nitro-benzene distils easily with steam and separates in the distillate as white prisms, some of which are perfectly formed. It melts at 83°.

The presence of nitrobenzene in the distillate can be recognized with certainty. Vigorously agitate the globules, when they have been separated as completely as possible from water, with granulated tin and a few c.c. of concentrated hydrochloric acid, until there is no odor of nitrobenzene. Pour the acid solution from undissolved tin, and add an excess of potassium hydroxide solution to decompose the double chloride of aniline and tin. Extract free aniline with ether. Withdraw the aqueous liquid from the separating funnel, and evaporate the ether extract spontaneously in air in a small glass dish. Aniline, formed by reducing nitrobenzene, will remain as globules which usually have a red or brown color. Dissolve these globules by shaking them vigorously with water, and use this solution for the hypochlorite and phenylisocyanide tests. A description of the former will be found under "Aniline" (page 36), of the latter under "Chloroform" (page 29).

ANILINE.



Aniline is a rather feeble base and its salts, when heated with a large quantity of water, are decomposed, at least in part, into aniline and the given acid. Aniline, therefore, if present, can always be detected in the distillate, even though the liquid distilled contain considerable tartaric acid. But the removal of aniline will not be complete, unless the solution before distillation be made alkaline with potassium or sodium hydroxide solution. Traces of aniline will remain dissolved in the distillate, whereas larger quantities will separate as globules. One part of aniline will dissolve in about 30 parts of water.

Aniline has a moderately vigorous, poisonous action. Doses of 1.5 to 2 grams, administered in the course of a day, have proved fatal to small dogs. It is not possible to state definitely the average lethal dose for human beings. Very serious results are said to have followed a dose of 3 or 4 grams of aniline (Kunkel, Toxikologie). Even inhalation of aniline vapor may cause severe, or fatal, intoxications. Aniline poisoning is attended by marked changes in the blood-pigment and in the blood-corpuscles. Formation of methaemoglobin, and the consequences resulting from it, are brought about by aniline. Even in the earliest stages of aniline poisoning, methaemoglobin can be detected by the spectroscope in blood taken from a human being during life. Like other blood poisons, aniline changes the shape of the corpuscles. The absolute number of the red blood-corpuscles is diminished, whereas the white cells are increased. The blood also contains considerably less oxygen, the percentage by volume being 5 to 10, as against 15 to 20, the normal quantity. Aniline can be detected in the blood.

It has been clearly demonstrated that aniline causes formation of methaemoglobin and destruction of red blood-corpuscles. In severer cases blood-pigment even appears in the urine. Aniline is found, as such, in the urine only in very minute quantities. The base is oxidized in the system to para-amidophenol ($C_6H_4.OH.NH_2(1,4)$). This is eliminated as a conjugate sulphuric acid, and probably as a conjugate glycuronic acid also. Experiments upon the dog have shown that, after a dose of aniline, the quantity of conjugate sulphuric acid eliminated increases in direct proportion to the quantity of aniline taken. Urine in aniline poisoning has a very dark color. Such urine is said almost always to reduce Fehling's solution, and it is a question whether this is caused by sugar, or by glycuronic acid.

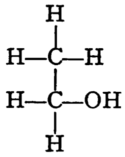
Aniline may be recognized by the following tests:

1. **Hypochlorite Test.**—Add a few drops of aqueous calcium or sodium hypochlorite solution to the distillate. A violet-blue, or purple-violet color will appear, if aniline be present. Avoid an excess of the reagent. The violet color gradually changes to a dirty red. This is a delicate test and will show aniline diluted 1 : 26,000.

2. **Bromine Water Test.**—This reagent, added to an aqueous aniline solution, will produce a flesh-colored precipitate. This test is sensitive 1 : 66,000.

3. Phenylisocyanide Test.—Heat an aniline solution with a few drops of chloroform and potassium hydroxide solution. The characteristic odor of phenylisocyanide will be recognized.

Ethyl Alcohol.



$$\begin{array}{l} \quad\; H \\ \quad\; | \\ H-C-H \\ \quad\; | \\ H-C-OH \\ \quad\; | \\ \quad\; H \end{array}$$

Ethyl alcohol distils easily with steam and, consequently, most of it will be in the first fraction. If present in sufficient quantity, it can be recognized in the distillate by its odor.

Fate of Alcohol in the Human Body.—All mucous membranes absorb alcohol very quickly and the greater part is consumed in the body. The uncertainty, formerly existing as to the fate of alcohol, has at last been removed by a series of experiments, the results of which have been accepted. It has been shown positively that only a small percentage of alcohol taken into the body is eliminated unchanged by the lungs (1.2 per cent.) and by the kidneys (1.6 per cent.). Strassmann[1] found the quantity eliminated by the lungs somewhat higher, as much as 6.4 per cent., and that by the kidneys 1.7 per cent. There is no reliable information, however, as to the place and manner in which alcohol is decomposed in the body. There is difference of opinion among toxicologists regarding alcoholic intoxication, as to whether the poison is distributed uniformly throughout the body, or accumulated in the brain in larger quantity than in other organs. The following percentages of alcohol were found in the organs of a man, who had died at the climax of severe, acute alcohol poisoning 18 hours after the last dose: liver 0.21, brain 0.47 and blood 0.33 per cent. It appears from these results, therefore, that the brain takes up an especially large quantity of alcohol. B. Fischer found the following quantities of alcohol in organs removed from a man, who had probably died from an overdose of alcohol:

Weight.	Organ.	Alcohol.
2720 grams	Stomach and intestines	30.6 grams.
2070 "	Heart, lungs and blood	10.85 "
1820 "	Kidneys and liver	7.8 "
1365 "	Brain	4.8 "

Ethyl alcohol may be recognized by the following tests:

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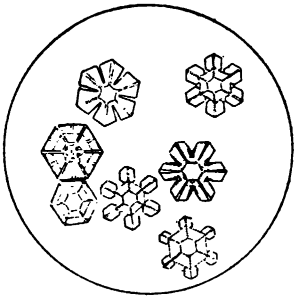
[1] *Pflüger's Archiv,* 49, 315 (1891).

1. **Lieben's Iodoform Test.**—Add a few c.c. of aqueous iodine-potassium iodide solution, or a small crystal of iodine, to the distillate and enough potassium hydroxide solution to give the liquid a distinct yellow to brownish color. Warm the solution very gently. If alcohol be present, a whitish to lemon-yellow precipitate of iodoform will soon appear. If the quantity of alcohol be very small the odor of iodoform will first be apparent, and then after some time a precipitate will form. When iodoform is deposited slowly, the crystals are very perfect hexagonal plates and stars (Fig. 7).

Fig. 7.



Iodoform Crystals.

This iodoform test is very delicate. It is not, however, characteristic of ethyl alcohol, because other primary alcohols, except methyl alcohol, and many secondary alcohols, as well as their oxidation products, aldehydes and ketones, give iodoform under the same conditions. Furthermore, acetic ether, aceto-acetic ether, lactic acid, cane-sugar, dextrin, proteïn substances and many other organic materials also give iodoform.

2. **Berthelot's Test.**—Vigorously agitate the liquid containing alcohol (distillate) with a few drops of benzoyl chloride and 3 to 5 drops of sodium hydroxide solution (10 per cent.), until the penetrating odor of benzoyl chloride is no longer present. The peculiar, aromatic odor of ethyl benzoate will be detected.

$$C_6H_5.COCl + C_2H_5.OH + KOH = C_6H_5.CO.OC_2H_5 + KCl + H_2O.$$

Ten c.c. of 0.5 per cent. alcohol will give a distinct odor of this ester. The detection of 0.05 grams of alcohol is certain by this test.

3. **Oxidation Test.**—Warm the liquid containing alcohol with dilute sulphuric, or hydrochloric acid, and add 1 or 2 drops of very dilute, aqueous potassium chromate solution. The color of the liquid will change from yellowish-red to green, and at the same time the odor of acetaldehyde will be recognized. This test is not characteristic of alcohol, because many other volatile organic compounds behave similarly.

4. **Vitali's Test.**—Thoroughly mix a few c.c. of distillate in a glass dish with a small piece of solid potassium hydroxide and 2 or 3 drops of carbon disulphide. Let this mixture stand for a short time without warming. When most of the carbon disulphide has evaporated, add a drop of ammonium molybdate solution and then an excess of dilute sulphuric acid. If the distillate contained alcohol, a red color will appear. Potassium xanthogenate is first formed.

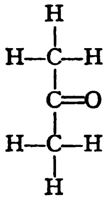
1. $CS_2 + C_2H_5.OH = HS.CS.OC_2H_5.$
2. $HS.CS.OC_2H_5 + KOH = KS.CS.OC_2H_5 + H_2O.$

This compound gives a red color with ammonium molybdate. The nature of this coloring-matter is unknown. Acetone and acetaldehyde produce a similar color. This test is given distinctly by 5 per cent. aqueous alcohol solution.

5. **Ethyl Acetate Test.**—Mix the liquid containing alcohol with the same volume of concentrated sulphuric acid. Add a very small quantity of anhydrous sodium acetate and heat. Ethyl acetate will be recognized by its odor.

1. $C_2H_5.OH + H_2SO_4 = C_2H_5O.SO_2.OH.$
2. $CH_3.CO.ONa + C_2H_5O.SO_2.OH = CH_3.CO.OC_2H_5 + NaHSO_4.$

ACETONE.



Human urine almost always contains a very small quantity of acetone. Under pathological conditions, especially in diabetes mellitus, urine contains much more. Moreover, it is frequently found in distillates from blood and various other organs, especially the liver, spleen, kidneys and brain. Distillates from parts of a cadaver frequently contain appreciable quantities of acetone.[1]

Acetone is a clear, colorless liquid which boils at 56°. It has a peculiar, fruity odor and is neutral in reaction. It is miscible in all proportions with water, alcohol and ether. It distils easily with steam. Practically all the acetone from any material will be in the first 20 c.c. of distillate. Acetone is not poisonous.

Acetone may be recognized by the following tests:

1. **Lieben's Iodoform Test.**—Add a few c.c. of aqueous iodine-potassium iodide solution, or a small crystal of iodine, to an aqueous solution of acetone (distillate) and then potassium hydroxide solution drop by drop until the color is yellow. Iodoform immediately separates, even in the cold, as a yellowish-white precipitate which is usually amorphous. Acetone differs from alcohol in giving iodoform, when ammonium hydroxide solution is substituted for potassium or sodium hydroxide solution (Gunning's acetone test).

1. $6KOH + 3I_2 = 3KI + 3KOI + 3H_2O.$
2. $CH_3.CO.CH_3 + 3KOI = CH_3.CO.CI_3 + 3KOH.$
3. $CH_3.CO.CI_3 + KOH = CHI_3 + CH_3.CO.OK.$

Acetaldehyde resembles acetone in giving iodoform in the cold and under conditions the same as those stated above.

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[1] This is the reason for giving so much prominence to acetone.

2. **Legal's Test.**—Add a few drops of freshly prepared sodium nitroprusside solution to a liquid containing acetone, and then potassium hydroxide solution. A red, or reddish-yellow color will appear. This color soon changes to yellow. Add an excess of acetic acid to the solution. The solution will now have a carmine to purplish-red color, according to the quantity of acetone present. Heat will change this color to violet. Acetaldehyde also gives Legal's test. The red color caused by aldehyde fades, upon addition of acetic acid, and changes to green with heat. Le Nobel states that ammonium hydroxide, or ammonium carbonate solution may be substituted for potassium hydroxide solution in Legal's test, but under these conditions the red color is very slow to appear. Le Nobel's modification, however, eliminates the possibility of confusing acetone with acetaldehyde.

Alcohol does not give Legal's test.

3. **Penzoldt's Indigo Test.**—Prepare a hot, saturated, aqueous ortho-nitro-benzaldehyde ($C_6H_4.NO_2.CHO(1,2)$) solution and allow it to cool. Add this solution to the liquid containing acetone, and also some sodium hydroxide solution. At first the color of the mixture is yellow. It then becomes green, and a blue precipitate of indigotin is formed in 10 to 15 minutes. When indigotin is present in traces only, shake the solution with chloroform. This solvent will dissolve the coloring-matter and become blue.

4. **Reynold's Test.**—Acetone will dissolve freshly precipitated mercuric oxide, and this test is based upon this property. Add mercuric chloride solution to the distillate, and an alcoholic potassium hydroxide solution. Shake thoroughly and filter. Add ammonium sulphide solution to the clear filtrate as an upper layer. If acetone be present, there will be a black zone (HgS) where the two solutions meet.

Detection of Acetone in Urine.—Acidify 200 to 500 c.c. of urine with a few drops of sulphuric acid and distil. Collect 20 to 30 c.c. of distillate. This will contain the entire quantity of acetone in the urine. Acetone thus obtained may possibly be derived from aceto-acetic acid which is often present in human urine, especially in a severe case of diabetes mellitus. Distillation decomposes this acid into acetone and carbon dioxide.

$$CH_3.CO.CH_2.CO.OH = CH_3.CO.CH_3 + CO_2.$$

Detection of Alcohol and Acetone in Mixtures.—Alcohol may be detected in presence of acetone by Berthelot's test. On the other hand, acetone may be distinguished from alcohol by Legal's or Penzoldt's test.

CARBON DISULPHIDE.

Carbon disulphide, CS_2, is a colorless liquid having a very characteristic odor. Its index of refraction is very high. It is only slightly soluble in water, 800 parts of water dissolving about 1 part of carbon disulphide. It is miscible in all proportions with absolute alcohol, ether, ethereal and fatty oils.

Carbon disulphide administered internally is rather a strong poison, causing especially decomposition of red blood-corpuscles. Even inhalation of carbon disulphide vapor frequently occasions severe poisoning. Such intoxications are not uncommon, because carbon disulphide is extensively used as a solvent in technical operations. It finds application in dissolving fats (for example, in removing fat from bones, crude wool, seeds and rags), in dissolving phosphorus in the match industry and in vulcanizing caoutchouc. In all these cases workmen breathe carbon disulphide vapor and its highly poisonous properties may cause severe intoxication.

Carbon disulphide distils very slowly with steam. Consequently, the second or third fraction of the distillate should be used in testing for this substance. If 40 c.c. be distilled from 100 c.c. of water containing 2 drops of carbon disulphide, the following 10 c.c. will give a distinct test.

Carbon disulphide may be recognized by the following tests:

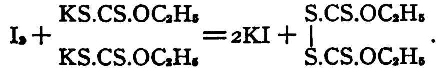
1. **Lead Acetate Test.**—Add a few drops of lead acetate solution to the liquid containing carbon disulphide (distillate). It will cause neither a precipitate (distinction between CS_2 and H_2S) nor a color. Add excess of potassium hydroxide solution and boil. A black precipitate (PbS) will appear. This is a very delicate test.

2. **Sulphocyanate Test.**—Heat an aqueous solution of carbon disulphide (distillate) for a few minutes with concentrated ammonium hydroxide solution and alcohol. Ammonium sulphocyanate will be formed. Concentrate this solution upon the water-bath to about 1 c.c. and acidify with dilute hydrochloric acid. Add a drop of ferric chloride solution and a deep red color will appear. This test will show even traces of carbon disulphide, for example 0.05 milligrams in 10 c.c. of water.

3. **Xanthogenate Test.**—Carbon disulphide may be converted into a salt of xanthogenic acid. Thoroughly shake a few c.c. of distillate for several minutes with 3 or 4 times its volume of saturated solution of potassium hydroxide in absolute alcohol. Faintly acidify the solution with acetic acid and add 1 or 2 drops of copper sulphate solution. If carbon disulphide be present, a brownish-black precipitate of cupric xanthogenate will appear. This will soon change to a yellow, flocculent precipitate of cuprous xanthogenate ($Cu_2(S.CS.OC_2H_5)_2$).

Quantitative Determination of Carbon Disulphide Vapor in Air.—Experiments have shown that air may be breathed without injurious results when it contains 0.5 to 0.8 milligrams of carbon disulphide per liter. Slight ill effects have been caused by 1.3 milligrams per liter after several hours. The effect of 3.4 milligrams per liter appeared in about 30 minutes, whereas 6 milligrams required 20 minutes. Larger doses, up to 10 milligrams per liter, soon occasion the first stages of paralysis (A. J. Kunkel, "Handbuch der Toxikologie," 1899). When carbon disulphide vapor is inhaled with air, 93 to 96 per cent. is again exhaled. Consequently, only a small percentage of the quantity inhaled is absorbed. The fate of this substance in metabolism has not been definitely determined. It is evident from the foregoing statements that the rooms of factories in which carbon disulphide is used should contain not more than 0.8 milligrams of this substance per liter.

The quantitative determination of carbon disulphide in air is made by drawing 10 to 20 liters of air, containing carbon disulphide vapor, through alcoholic potassium hydroxide solution in a Peligot absorption-tube. Carbon disulphide will be converted quantitatively into potassium xanthogenate. Titrate this in neutral solution with N/10 iodine solution.

1. 

$$I_2 + \begin{array}{l} KS.CS.OC_2H_5 \\ KS.CS.OC_2H_5 \end{array} = 2KI + \begin{array}{l} S.CS.OC_2H_5 \\ | \\ S.CS.OC_2H_5 \end{array}.$$

Dilute the contents of the receiver at the end of the experiment with 96 per cent. alcohol and bring the volume to 50 c.c. Measure an aliquot portion of this solution and dilute with water. Faintly acidify the solution with acetic acid and remove excess of acid with calcium carbonate, or better with acid sodium carbonate. Add starch solution and N/10 iodine solution until there is a permanent blue color. Iodine converts xanthogenic acid into xanthogenic persulphide. The equation above shows that 1 c.c. of N/10 iodine solution corresponds to

$$\frac{CS_2}{10000}\text{ grams} = \frac{76}{10000} = 0.00076 \text{ grams of } CS_2.$$

E. Rupp and L. Krauss[1] consider that the action of iodine upon potassium xanthogenate is expressed by the following equation:

$$2.\quad 2KS.CS.OC_2H_5 + H_2O + 2I = KS.CS.SK + 2C_2H_5.OH + 2HI + S.$$

The abundant separation of sulphur during the reaction seems to substantiate this view. It is evident that 1 molecule of iodine corresponds to 2 molecules of xanthogenate in both equations. Consequently, the factor for N/10 iodine solution in terms of carbon disulphide is not at all changed by the manner of writing the reaction. This method is applicable to the quantitative determination of xanthogenates also.

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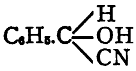
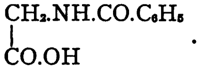
[1] *Berichte der Deutschen chemischen Gesellschaft,* 35, 4157 (1902).

BITTER ALMOND WATER AND BENZALDEHYDE.

Bitter almond water (Aqua Amygdalae Amarae of the Pharmacopoeia) contains hydrocyanic acid. Only a small portion of this acid, however, is free so that it can be precipitated by silver nitrate solution. The greater part is combined as the cyanohydrin of benzaldehyde,  $C_6H_5.C\begin{cases}H\\OH\\CN\end{cases}$, which does not react with silver nitrate. But potassium hydroxide solution will decompose this compound.

$$C_6H_5.CH(OH)CN + KOH = KCN + H_2O + C_6H_5.CHO.$$

The "Pharmacopoeia" prescribes addition of potassium hydroxide solution to cause this decomposition and also to neutralize free nitric acid formed in titration with silver nitrate.

Pure benzaldehyde, also called hydrocyanic acid-free oil of bitter almonds, is not poisonous. It is oxidized to benzoic acid in the body and most of it is eliminated in the urine as hippuric acid,  $\begin{matrix}CH_2.NH.CO.C_6H_5\\|\\CO.OH\end{matrix}$. Ordinary commercial oil of bitter almonds contains hydrocyanic acid and is poisonous in proportion to the quantity of this acid present. Benzaldehyde and hydrocyanic acid are combined chemically as the cyanohydrin of benzaldehyde. Test for hydrocyanic acid by thoroughly shaking about 2 c.c. of oil of bitter almonds with 20 c.c. of potassium hydroxide solution and making the Prussian blue test. When oil of bitter almonds is mixed with other material, distil with steam from a solution acidified with tartaric, or dilute sulphuric acid, and test the first part of the distillate for hydrocyanic acid. If benzaldehyde be present, the distillate at the same time will be milky and have the characteristic odor of this compound. Distil until the drops of water are perfectly clear.* Benzaldehyde may be detected with certainty, and at the same time distinguished from nitrobenzene which has a somewhat similar odor, by adding a few drops of potassium hydroxide solution to the milky distillate, to combine with any hydrocyanic acid, and extracting with ether. The ether upon evaporation will deposit benzaldehyde as globules, which can be positively identified by conversion into benzoic acid. Heat the globules for a few minutes in a small flask, attached to a reflux condenser, with about 10 c.c. of potassium dichromate solution and a small quantity of dilute sulphuric acid. Cool, extract with ether and evaporate the ether solution in a glass dish. When the material contains benzaldehyde, this residue will consist of benzoic acid. This substance may be further identified by its melting point (120–121°), its property of subliming and the test with ferric chloride solution.[1]

SYNOPSIS OF GROUP I.

The material to be examined must first be rendered uniform by grinding or chopping. Add sufficient water to thin the mass, acidify with tartaric acid and distil. It is advisable to collect the distillate in two or three fractions. Test the first 5 to 10 c.c. of distillate for hydrocyanic acid, chloroform, ethyl alcohol, acetone and possibly also for nitrobenzene and iodoform. Use the remainder of the distillate in testing for the other volatile poisons.

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[1] Dissolve the residue in a small quantity of water, and neutralize benzoic acid by heating the solution to boiling with excess of calcium carbonate. Filter and add a few drops of ferric chloride solution. If benzoic acid be present, a flesh-colored precipitate of basic ferric benzoate will appear. Tr.

Phosphorus: Scherer's test. Phosphorescence during distillation in a dark room. Evaporate distillate with strong chlorine water and test for phosphoric acid, or examine distillate by Blondlot-Dussard method.

Hydrocyanic Acid: Odor. Schönbein's test. Prussian blue test. Sulphocyanate test. Nitroprusside test. Silver nitrate test.

Carbolic Acid: Odor. Red color with Millon's reagent. Yellowish-white precipitate with bromine water. Violet color with ferric chloride solution.

Chloroform: Separation of colorless globules, when the quantity is large. Odor. Phenylisocyanide test, when heated with aniline and potassium hydroxide solution. Reduces silver nitrate and Fehling's solutions with heat. Red color with resorcinol and potassium hydroxide solution. Blue color with naphthol and potassium hydroxide solution.

Chloral Hydrate: Gives chloroform reactions. Brick-red precipitate with Nessler's solution which in time becomes yellowish-green. Gives chloroform and magnesium formate, when heated with magnesium oxide and water. Test for formate with silver nitrate or mercuric chloride solution.

Iodoform: Odor. Distillate milky and yellowish-white. Ether extract of distillate leaves crystals upon evaporation, gives chloroform reactions.

Nitrobenzene: Yellowish globules with characteristic odor. Reduced to aniline, when shaken with tin and hydrochloric acid. Test for aniline.

Aniline: Violet color with calcium hypochlorite solution. Phenylisocyanide test, when heated with chloroform and potassium hydroxide solution. Flesh-colored precipitate with bromine water.

Ethyl Alcohol: Iodoform test. Odor of ethyl benzoate, when shaken with benzoyl chloride and sodium hydroxide solution. Green color, when heated with potassium dichromate and hydrochloric acid.

Acetone: Gives iodoform, even in the cold, with iodine and potassium hydroxide or ammonium hydroxide solution. Legal's test. Indigotin test. Reynold's test.

Carbon Disulphide: Black precipitate, or only black coloration (PbS), when heated with lead acetate and potassium hydroxide solutions. Formation of ammonium sulphocyanate by evaporation with concentrated ammonium hydroxide solution and detection with ferric chloride solution. Formation of potassium xanthogenate, when shaken with alcoholic solution of potassium hydroxide and detection with copper sulphate solution.

CHAPTER II.

NON-VOLATILE ORGANIC SUBSTANCES—GROUP II.

Members of Group II are not volatile, when an acid solution is distilled with steam. This group comprises alkaloids, glucosides, certain synthetic organic drugs and other similar compounds.

Put a portion of finely chopped material into a large flask, and thoroughly mix with two or three times the quantity of absolute alcohol.[1] Add enough tartaric acid solution to give the mixture a distinct acid reaction after it has been shaken. Laboratory experiments usually require 20 to 30 drops of 10 per cent. tartaric acid solution. Avoid much of an excess of tartaric acid, since it may act as an objectionable impurity in the ether extract, owing to its solubility in this solvent. Connect the flask, which should be about half-full, with a glass tube (80 to 100 cm. long) serving as a reflux cooler. Frequently shake and heat 10 to 15 minutes upon the water-bath. In the extraction of a large quantity of material from a cadaver, connect the flask with an upright Liebig condenser used as a reflux cooler (Fig. 8). Cool the flask contents and filter. Wash the residue with alcohol. Evaporate the filtrate, which must have an acid reaction, to a thin syrup in a glass dish upon the water-bath. Thoroughly mix this residue with 100 c.c. of cold water. Usually this causes an abundant separation of fat and resinous matter, especially when parts of a cadaver are examined. Filter and evaporate the filtrate to dryness, or to a syrup, upon the water-bath. Thoroughly mix this residue with absolute alcohol. As the result of this treatment, a whitish substance, which is more or less viscous or slimy, usually remains undissolved. This residue, which consists chiefly of proteïn substances (albumin, albumoses and peptones), dextrin-like compounds and in part also of inorganic salts, frequently becomes granular upon standing. Alkaloids and other organic poisons are dissolved. The larger the quantity of absolute alcohol used, the more complete is the precipitation of those substances which interfere more or less with the detection of organic poisons. Again evaporate the filtered, alcoholic solution upon the water-bath, and dissolve the residue in about 50 c.c. of water. If the solution be not perfectly clear, filter through a moistened paper.

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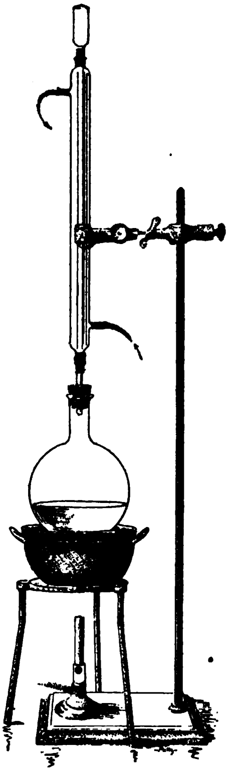
[1] Commercial alcohol usually contains basic compounds, the presence of which is objectionable. They should be removed by adding tartaric acid to the alcohol and distilling. Alcohol should not be used in toxicological analysis, unless an actual test has shown it to be free from such impurities. Tr.

Fig. 8.



Liebig Condenser as Reflux Cooler.

The result of this procedure is a solution containing alkaloidal tartrates and other organic substances belonging to this group. This solution should have an acid reaction and should be practically free from proteïn substances, fat, resinous bodies and coloring-matter. If the solution fulfil these requirements, it is ready to be examined for organic poisons according to the "Stas-Otto" method. The utmost care must be taken in preparing this solution, because definite conclusions cannot be drawn from the uncertain tests given by impure material.

When the material is a powder mixed with cane-sugar, it is usually possible, after the aqueous solution has been acidified with tartaric acid, to extract directly with ether and to continue according to the Stas-Otto method. Ordinary and even absolute alcohol will dissolve considerable cane-sugar. Consequently, this substance cannot be entirely excluded by alcohol.

A. EXTRACTION OF THE ACID SOLUTION WITH ETHER.

Thoroughly extract the acid, aqueous solution two or three times with ether, using each time about the same quantity of solvent. Employ a separating funnel for this purpose (Fig. 9). Pour the combined ether extracts into a dry flask which is loosely stoppered. If the solution be allowed to stand for 1 or 2 hours at perfect rest, a few drops of water will usually collect on the bottom of the flask. Decant the ether solution and pour through a dry filter. Slowly evaporate the ether solution in a small glass dish upon a water-bath previously heated slightly above 35°. Do not have gas burning during this operation! Examine the residue in the manner to be described later. An excellent method of evaporating ether consists in setting a small glass dish (8 to 10 cm. in diameter) upon a hot water-bath and in dropping the filtered ether extract into it as fast as the solvent evaporates. In this way a large quantity of extract may be evaporated in a small dish. The advantage of this method is the ease with which the residue can be removed for the various tests. The residue is usually quite small and it is not advisable to have it distributed over too large a surface.

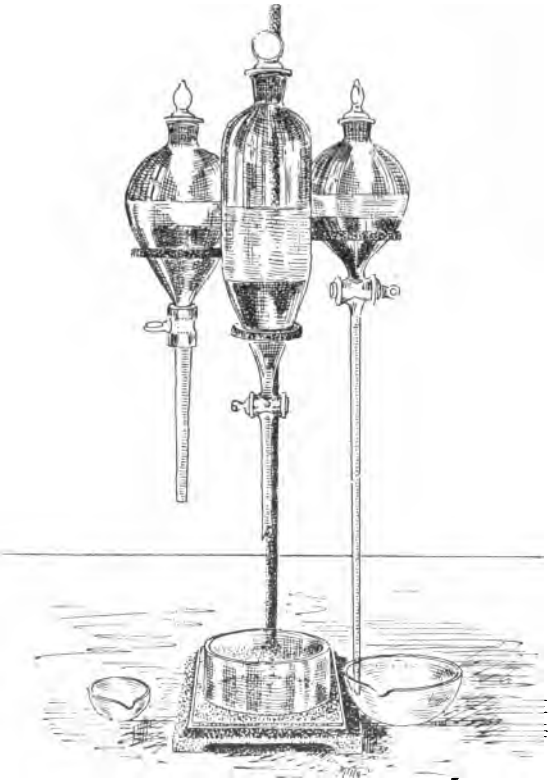
Examine the residue from the ether extract for the following substances:

Picrotoxin
Colchicin

Acetanilide
Phenacetine

Picric Acid **Antipyrine**
Caffeïne **Salicylic Acid**
Mercuric Cyanide.[1]

FIG. 9.



Separating Funnels and Glass Crystallizing Dishes.

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[1] Ether to some extent will extract mercuric cyanide from a tartaric acid solution which is not too dilute. For instance, it will remove appreciable quantities from 100 c.c. of 0.1 per cent. mercuric cyanide solution, but the extraction will not be complete. The solution after five extractions will still give a distinct test for mercury. Ether will remove not even a trace of mercuric cyanide from 0.01 per cent. solution.

First, note the general appearance and taste of the residue. Then examine it with a microscope. Very definite conclusions as to the presence or absence of certain substances can frequently be drawn. A very bitter residue should be examined carefully for picrotoxin and colchicin. If there be a pronounced yellow color, the examination should include picric acid also. A tasteless, or only faintly bitter, residue probably does not contain these substances and should be examined for acetanilide, antipyrine, caffeïne, phenacetine and salicylic acid.

The residue from evaporation of the ether extract may contain the following substances:

Picrotoxin: Usually a thick syrup which gradually solidifies and becomes crystalline. Tastes intensely bitter.

Colchicin: Yellowish, amorphous residue which does not become crystalline. Tastes intensely bitter. Dissolves in water with a yellowish color.

Picric Acid: Residue intensely yellow. Tastes bitter. Usually appears as a syrup which gradually solidifies and becomes crystalline.

Acetanilide: Leaflets or flattened needles. Has a faint, burning taste but is not bitter.

Antipyrine: Residue a syrup which is rarely crystalline. Tastes mildly bitter.

Caffeïne: Residue composed of shining needles frequently in radiating clusters. Tastes mildly bitter.

Phenacetine: Inodorous and tasteless leaflets and small needles.

Salicylic Acid: Crystallizes frequently in long needles. Tastes harsh and at the same time sweet and acid.

To test for cyanide, add ammonium sulphide solution to the ether residue. This will precipitate mercuric sulphide and the filtrate will contain ammonium sulphocyanate (see hydrocyanic acid, page 18). Ether to some extent will also remove mercuric chloride from an aqueous solution.

If the ether contain much tartaric acid, the residue will have a strong acid taste. This and other acids (for example, lactic acid) may keep otherwise easily crystallizable substances in a syrupy condition.

PICROTOXIN.

Picrotoxin, $C_{15}H_{16}O_6.H_2O$ or $C_{30}H_{34}O_{13}$, crystallizes in long, colorless needles which dissolve with difficulty in cold water but more readily in hot water and alcohol. It is slightly soluble in ether, freely soluble in chloroform and amyl alcohol and practically insoluble in benzene and petroleum ether. Picrotoxin is easily decomposed. Even boiling with benzene breaks this compound into picrotoxinin and picrotin.

$$\underset{\text{Picrotoxin}}{C_{30}H_{34}O_{13}} = \underset{\text{Picrotoxinin}}{C_{15}H_{16}O_6} + \underset{\text{Picrotin}}{C_{15}H_{18}O_7}(?).$$

Chloroform produces the same result more quickly and even in the cold. Picrotin occurs with picrotoxin in the kernel of Cocculus indicus.

Dilute acids do not increase the solubility of picrotoxin. It gives an intense orange-red color with concentrated sulphuric acid. But it behaves like an acid with strong bases. Potassium, sodium and ammonium hydroxides dissolve it quite easily, forming non-crystalline, unstable salts. Dissolved in dilute potassium hydroxide solution, picrotoxin is completely decomposed even in two hours.

Picrotoxin may be recognized by the following tests:

1. **Fehling's Solution Test.**—Dissolve not too small a quantity of picrotoxin in about 2 c.c. of water containing sodium hydroxide. Add a few drops of Fehling's solution and warm but do not shake. A red or yellowish-red precipitate forms and settles to the bottom. If the ether residue, of which not too small a quantity should be taken, fail to give a clear solution in very dilute sodium hydroxide solu-

tion, filter through moistened paper and examine the filtrate with Fehling's solution.

2. **Silver Nitrate Test.**—Warm picrotoxin with aqueous silver nitrate solution containing a slight excess of ammonium hydroxide solution. The reducing action of picrotoxin will produce a black precipitate of metallic silver, or a dark brown color when only traces are present.

3. **Langley's Test.**—Mix picrotoxin with about three times the quantity of potassium nitrate, and moisten the mixture with pure, concentrated sulphuric acid. Then add strong sodium hydroxide solution in excess and an intense red color will appear.

4. **H. Melzer's[1] Test.**—Put some picrotoxin upon a watch-glass and add 1 or 2 drops of a mixture of benzaldehyde and absolute alcohol. Careful addition of a drop of concentrated sulphuric acid will produce a distinct red color. If the watch-glass be tilted, red streaks will run from the substance through the liquid. Use preferably a freshly prepared, 20 per cent. solution of benzaldehyde in absolute alcohol. Benzaldehyde alone gives a yellowish-brown color with concentrated sulphuric acid, and alcohol is added as a diluent to diminish this color as much as possible. Under these conditions the solution has a light yellow color, and the dark red tint caused by picrotoxin is very beautifully defined. This red color is very unstable, as is also the yellow color of benzaldehyde and sulphuric acid, and, beginning at the margin, it gradually fades into a pale pink or violet. H. Kreis[2] has found that cholesterine and phytosterine[3] give similar colors with Melzer's reagent.

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[1] *Zeitschrift für analytische Chemie*, 37, 351 and 747 (1898).

[2] *Chemiker-Zeitung*, 33, 21 (1899).

[3] A substance very similar to cholesterine, and named paracholesterine or phytosterine, is found in the seeds of certain plants. (Perkin and Kipping, *Organic Chemistry*, page 608.)

Detection of Picrotoxin in Beer.—First, neutralize the beer with magnesium oxide. Then evaporate 500 c.c. or more to a syrup upon the water-bath. Digest this residue with 4 or 5 times its volume of alcohol and evaporate the alcoholic extract. Dissolve the residue in hot water and filter the solution through a moistened paper. Acidify the filtrate with dilute sulphuric acid and extract repeatedly with ether, or better with chloroform. Evaporate these extracts and test the residue for picrotoxin.

COLCHICIN.

Colchicin, $C_{22}H_{25}NO_6$, which is poisonous and very bitter to the taste, usually appears as a yellowish, amorphous powder. It is freely soluble in water, alcohol, benzene and chloroform, less so in ether, and almost insoluble in petroleum ether. Solutions of colchicin have a more or less yellowish color which becomes more pronounced upon addition of acids or alkalies. Ether or chloroform will extract colchicin from an acid, aqueous solution. Upon evaporation of the solvent, colchicin will appear as a yellowish, sticky residue resembling a resin or varnish. Heated with water containing sulphuric acid, colchicin splits into colchiceïn and methyl alcohol.

$$\underset{\textbf{Colchicin}}{C_{22}H_{25}NO_6} + H_2O = \underset{\textbf{Colchiceïn}}{C_{21}H_{23}NO_6} + \underset{\textbf{Methyl Alcohol}}{CH_3.OH.}$$

On the other hand, colchicin is formed when colchiceïn is heated to 100° with sodium methylate ($CH_3.ONa$) and methyl iodide ($CH_3.I$). The most probable formula of colchicin, is $(CH_3.O)_3C_{15}H_9(NH.C_2H_3)CO.OCH_3$.

Colchicin may be recognized by the following tests:

1. **Tannic Acid Test.**—This reagent will precipitate colchicin from aqueous solution, if not too dilute, as white flocks. This test, however, is not characteristic of colchicin.

2. **Sulphuric-Nitric Acid Test.**—Concentrated sulphuric acid dissolves colchicin with a yellow color which a drop of nitric acid will change to green, blue, violet, wine-red and finally back to yellow.

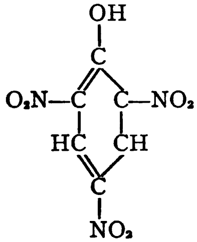
3. **Nitric Acid Test.**—Concentrated nitric acid dissolves colchicin with a dirty violet color which soon changes, when stirred, to brownish-red and finally to yellow. Addition of dilute sodium or potassium hydroxide solution, until the reaction is alkaline, produces a beautiful orange-yellow or orange-red color. This is the test by which colchicin can be identified.

4. **Test with Erdmann's Reagent.**—This reagent will dissolve colchicin with a blue color.

It is stated that substances resembling colchicin may occur in beer and also in putrefying cadavers.

Purification of the Residue Containing Colchicin.—To isolate as pure colchicin as possible from the yellow residue, extract with warm water. Filter the solution and, when cold, extract it first with petroleum ether. This will remove fatty or resinous, colored impurities but not colchicin. Then extract with chloroform. Or precipitate colchicin from aqueous solution, which must not be too dilute, with tannic acid. Collect this precipitate upon a filter and wash with cold water. Mix the moist precipitate with freshly precipitated, washed lead hydroxide. Dry the mixture, grind to a powder and extract with chloroform. Evaporation of the solvent will leave nearly pure colchicin.

PICRIC ACID.



Picric acid, or 2,4,6-trinitrophenol, crystallizes in yellow, rhombic prisms. It is very bitter to the taste. Though soluble in cold water only in small quantity (1 : 160), picric acid dissolves quite readily in hot water, alcohol, ether and benzene. A distinguishing feature of this substance is the intensely yellow color which it imparts to material containing it and to aqueous, alcoholic and ether extracts.

Picric acid is quite a vigorous poison. Administered by the mouth, it produces a distinct yellow color first in the conjunctiva. Then this extends to the entire skin and is usually called "picric acid icterus."

For some time the urine has a ruby-red color, owing to presence of picraminic acid ($C_6H_2.OH(NH_2)(NO_2)_2(1,2,4,6)$), formed by reduction of picric acid in the system. Moreover, the urine is acid, clear and free from albumin and bile constituents. Picric acid and its salts like most nitro-compounds decompose blood-corpuscles and form methaemoglobin. Finally it acts as a precipitant of albumin. This is noticeable especially in those organs of the body which have an acid or only a faintly alkaline reaction.

Picric acid may be recognized by the following tests:

1. **Dyeing Test.**—Dissolve the substance containing picric acid in hot water and put a white, woolen (or silken) and a cotton thread into this solution. In a few hours (12 to 24) remove the threads and thoroughly rinse in pure water. If picric acid be present, the woolen or silken, but not the cotton thread will be dyed yellow. In other words, picric acid is not fast upon vegetable fibres like cotton. Picric acid, diluted 1:110,000, will still produce a yellow color upon wool.

2. **Isopurpuric Acid Test.**—Gently heat an aqueous solution of picric acid with a few drops of saturated, aqueous potassium cyanide solution and 2 drops of sodium hydroxide solution. The solution will be colored red owing to formation of potassium isopurpurate. One milligram of picric acid, dissolved in 5 c.c. of water, will give a distinct test.

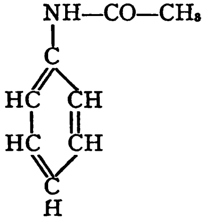
3. **Picraminic Acid Test.**—Heat picric acid solution with a few drops of sodium hydroxide solution and glucose. Picraminic acid, formed by reduction of picric acid, colors the solution deep red. Avoid excess of sodium hydroxide solution, otherwise there will be a red color due solely to the action of the alkali upon glucose.

The test may also be made by warming picric acid solution with a few drops of sodium hydroxide and ammonium sulphide solutions. This will reduce picric acid and produce a red color.

4. **Ammoniacal Copper Sulphate Test.**—Add a few drops of ammoniacal copper sulphate solution to an aqueous

picric acid solution. A yellowish-green precipitate, consisting of needle-like crystals with a polarizing action upon light, will appear. Picric acid, diluted 1:80,000, will give this test.

ACETANILIDE.



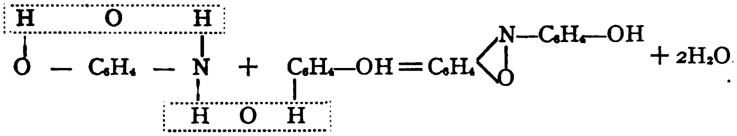
Acetanilide crystallizes in colorless and inodorous, shining leaflets. It has a faint, burning taste. It melts at 113 to 114°. It is soluble in 230 parts of cold water, in about 22 parts of boiling water and in 35 parts of alcohol. It is freely soluble in ether and still more so in chloroform. All acetanilide solutions are neutral. Heated to boiling with potassium hydroxide solution, acetanilide is decomposed into aniline, the vapor of which has an aromatic odor, and potassium acetate.

$$C_6H_5.NH.CO.CH_3 + KOH = C_6H_5.NH_2 + CH_3.CO.OK.$$

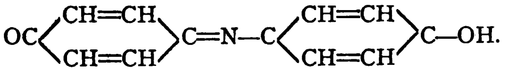
Acetanilide may be recognized by the following tests:

1. **Indophenol Test.**—Boil acetanilide with about 2 c.c. of concentrated hydrochloric acid. Cool and add 2 to 4 c.c. of saturated, aqueous carbolic acid solution. A few drops of calcium hypochlorite solution will produce a dirty violet-red color. In time the color will become deeper, especially if the mixture be thoroughly shaken. Then carefully add ammonium hydroxide solution as a surface-layer. This upper layer will assume a beautiful indigo-blue color.

In this test hydrochloric acid decomposes acetanilide into aniline and acetic acid. Calcium hypochlorite oxidizes aniline to para-amidophenol ($C_6H_4.OH(NH_2)(1,4)$) which, in presence of an oxidizing agent, combines with phenol to form an indophenol.



Very likely there is a shifting of the bond between oxygen and nitrogen which would give indophenol the "quinoidal form"

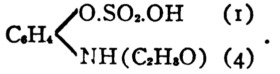


Phenacetine also gives the indophenol test.

2. **Phenylisocyanide Test.**—Boil acetanilide with a few c.c. of potassium hydroxide solution. Then add 2 or 3 drops of chloroform and again heat. The very offensive odor of phenylisocyanide will be developed.

3. **Calcium Hypochlorite Test.**—Acetanilide, if boiled a few minutes with alcoholic potassium hydroxide solution, will be completely decomposed. Dilute the product of the reaction with water and extract aniline with ether. This solvent upon evaporation will deposit aniline as an oily liquid. Dissolve the latter in water and test with calcium hypochlorite.

Detection of Acetanilide in Urine[1] by the Product of Its Metabolism.—Scarcely more than traces of unaltered acetanilide appear in urine even after large doses. The most essential change occurring in the body is oxidation of the benzene ring which produces aceto-para-amidophenol. This forms a conjugate sulphuric acid and appears in the urine as a salt of aceto-para-amidophenyl sulphuric acid,



$$C_6H_4 \begin{cases} O.SO_2.OH & (1) \\ NH(C_2H_3O) & (4) \end{cases}.$$

To some extent also, a conjugate glycuronic acid of aceto-para-amidophenol is formed. These compounds, heated with concentrated hydrochloric acid, give para-amidophenol which can be detected by the indophenol test. Such urine, boiled a few minutes with concentrated hydrochloric acid, will usually give this test. But the test will be more certain, if para-amidophenol be first isolated. Boil a larger quantity of urine (300 to 500 c.c.) a few minutes with about 10 c.c. of concentrated hydrochloric acid. Then add an excess of sodium carbonate and repeatedly extract the cool urine with large quantities of ether. Distil or evaporate the ether. Para-amidophenol usually appears as a reddish or brownish oil. An aqueous solution of this substance will give a beautiful indophenol test.

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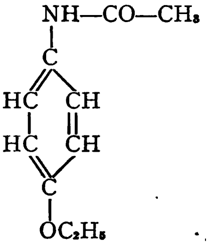
[1] To study the behavior of acetanilide in the body, take at night 0.3 grams of this substance at a dose twice in the course of an hour, and examine the urine passed in the next twelve hours.

PHENACETINE.

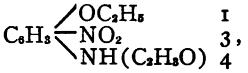


Phenacetine crystallizes in shining leaflets which are without color, odor or taste. It melts at 134 to 135°. Phenacetine is soluble in about 1400 parts of cold water, 70 parts of boiling water and 16 parts of alcohol. It is freely soluble in ether and chloroform. Its solutions are neutral. Concentrated sulphuric acid dissolves it without color. Phenacetine is very closely related to acetanilide but does not give the phenylisocyanide test.

Phenacetine may be recognized by the following tests:

1. **Chromic Acid Test.**—Dissolve phenacetine in 2 or 3 c.c. of concentrated hydrochloric acid and boil one minute. Dilute with 10 to 20 c.c. of water and filter when cold. A few drops of chromic acid solution added to the filtrate will gradually produce a ruby-red color.

2. **Autenrieth-Hinsberg Test.**[1] (*a*) **With Dilute Nitric Acid.**—Heat phenacetine to boiling with a few c.c. of dilute nitric acid (10 to 12 per cent.). It is soluble and gives an intense yellow to orange-red color. As the solution cools, if sufficiently concentrated, nitro-phenacetine,



$$C_6H_3\begin{cases}OC_2H_5 & 1\\ NO_2 & 3,\\ NH(C_2H_3O) & 4\end{cases}$$

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[1] *Archiv der Pharmacie,* Band 229, 456 (1891).

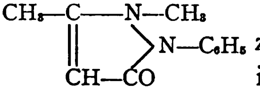
will crystallize in long, yellow needles which melt at 103°. This test is delicate, and characteristic of phenacetine. It serves to distinguish phenacetine from acetanilide and antipyrine, both of which give colorless solutions when warmed with dilute nitric acid.

(*b*) **With Concentrated Nitric Acid.**—A few drops of concentrated nitric acid poured upon phenacetine produce a yellow to orange-red color. Part of the phenacetine is dissolved with the same color and heat completes the solution. Nitro-phenacetine crystallizes as the solution cools.

3. **Indophenol Test.**—Boil phenacetine 1 or 2 minutes with about 2 c.c. of concentrated hydrochloric acid. Dilute with water and add a few c.c. of aqueous carbolic acid solution. Filter the solution when cold. If a few drops of freshly prepared calcium hypochlorite solution be added, the filtrate will have a beautiful, carmine-red color. Addition of ammonium hydroxide solution in excess will change this color to violet-blue. Freshly prepared chlorine water, or 3 per cent. chromic acid solution, may be substituted for hypochlorite solution. Chromic acid gives more of a ruby-red color. Since acetanilide also gives the indophenol test, the nitric acid test is more reliable in identifying phenacetine.

"Phenacetine urine" usually gives a beautiful indophenol test. When such urine is boiled with concentrated hydrochloric acid, para-amidophenol and para-amidophenetol are formed.

ANTIPYRINE.



Antipyrine, or dimethyl-phenyl-pyrazolone, appears in tabular crystals having a very faint, bitter taste and melting at 113°. One part of antipyrine is soluble in less than 1 part of cold water, in 1 part of alcohol, 1 part of chloroform and about 50 parts of ether. An aqueous antipyrine solution has a neutral reaction, although this compound is a base which forms crystallizable salts with acids. Ether extracts only small quantities of antipyrine from a solution containing much tartaric acid. Ether, or better chloroform, extracts by far the greater part of the antipyrine when the solution has been made alkaline. Consequently, in the Stas-Otto process, antipyrine will appear in ether extracts A and B, and under certain circumstances even in the chloroform extract of the ammoniacal solution. In testing for antipyrine alone, dissolve the substance in water and make the solution alkaline with sodium hydroxide solution. Repeatedly extract this solution with chloroform instead of with ether.

Antipyrine may be recognized by the following tests:

1. **Tannic Acid Test.**—Tannic acid solution produces an abundant, white precipitate, when added to an aqueous antipyrine solution.

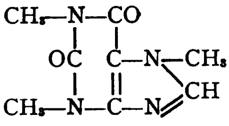
2. **Fuming Nitric Acid Test.**—Dissolve antipyrine in a few drops of water and add 1 or 2 drops of fuming nitric acid. The solution will be colored green. If this solution be heated to boiling, another drop of this acid will produce a red color. Two c.c. of antipyrine solution (1:200) will give this test distinctly.

3. **Nitrite Test.**—Add a few drops of potassium or sodium nitrite solution to an aqueous antipyrine solution and then dilute sulphuric acid. A green or blue color will appear. A few drops of acetic acid may be substituted for sulphuric acid but the solution must be heated.

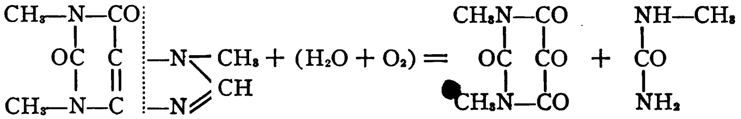
4. **Ferric Chloride Test.**—Add 1 or 2 drops of ferric chloride solution to an aqueous antipyrine solution. It will produce a deep red color which dilute sulphuric acid will change to light yellow.

In testing for antipyrine in the ether residue, dissolve in a few drops of water and apply the ferric chloride and fuming nitric acid tests to this solution.

CAFFEÏNE.



Caffeïne, or 1,3,7-trimethyl-2,6-dioxypurine (also called theïne), crystallizes in white shining needles. It is soluble in 80 parts of water, giving a colorless solution with a neutral reaction and a faint, bitter taste. Caffeïne is quite easily soluble in hot water. It requires for solution nearly 50 parts of alcohol, only 9 parts of chloroform and is only slightly soluble in ether. In crystallizing from hot water caffeïne combines with 1 molecule of water, a part of which it loses upon exposure to air and all when dried at 100°. Caffeïne melts at 230°, but somewhat above 100° it begins to volatilize in small quantity and at 180° to sublime without leaving a residue. Concentrated sulphuric and nitric acids dissolve it without color. Caffeïne is a very weak base and its salts are decomposed by water. The relation existing between caffeïne and uric acid is quite apparent when the products, formed by oxidizing these two substances with potassium chlorate and hydrochloric acid, are compared. Oxidation of uric acid yields alloxan and urea; caffeïne gives dimethyl-alloxan and monomethyl-urea.



Caffeine. Dimethyl-alloxan. Monomethyl urea.

Since caffeïne is only slightly soluble in ether, its aqueous solutions must be repeatedly extracted with large quantities of this solvent. But even then, considerable caffeïne will remain in the aqueous solution. When the solution is made alkaline, ether will extract a further portion of caffeïne and finally some will be extracted by chloroform from the ammoniacal solution. By the evaporation of these extracts caffeïne is usually deposited in concentric clusters of long, shining needles.

Caffeïne may be recognized by the following tests:

1. **Tannic Acid Test.**—This reagent, added to an aqueous caffeïne solution, causes a heavy precipitate which is soluble in an excess of the acid. This test is not characteristic of caffeïne.

2. **Oxidation Test.**—Pour a few c.c. of saturated chlorine water[1] over caffeïne and evaporate the solution to dryness upon the water-bath. A yellowish-red residue will remain. If a few drops of ammonium hydroxide solution be added, a beautiful, purple-red color will immediately appear. Potassium or sodium hydroxide solution gives more of a blue-violet color.

As in the murexide test, oxidation may be brought about by pouring a few drops of concentrated nitric acid over caffeïne in a porcelain dish and evaporating to dryness upon the water-bath. A yellow spot of amalinic acid will remain. Ammonium hydroxide solution will dissolve this substance with a purple color.

The elimination and fate of caffeïne in animal metabolism have been subjects of considerable study. The results show that only a small part of the caffeïne administered passes through the body unchanged and appears in the urine. The greater part is decomposed and the methyl groups are first split off. Consequently, the nitrogen of caffeïne is eliminated mostly as urea. Monomethyl-xanthine, which is always present to some extent after administration of caffeïne, is an interesting, intermediate decomposition product.

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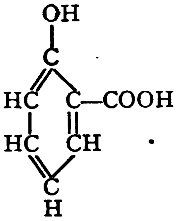
[1] A convenient method of preparing a saturated, aqueous chlorine solution is to heat potassium chlorate with hydrochloric acid and pass the chlorine into a small quantity of water.

SALICYLIC ACID.



Salicylic acid crystallizes in long, white needles which dissolve in about 500 parts of cold water and in 15 parts of boiling water. The acid is freely soluble in alcohol, ether, chloroform and carbon disulphide. It has a peculiar taste which may be described as sweetish, acidulous and rather acrid. It melts at about 157°. If heated carefully, salicylic acid will sublime in fine needles without decomposition. A small quantity of the acid may show this behavior even under the water-bath. In steam distillation a small quantity of the acid is volatilized. If heated quickly, salicylic acid is decomposed in part into phenol and carbon dioxide.

Salicylic acid may be recognized by the following tests:

1. **Ferric Chloride Test.**—Addition of ferric chloride solution to an aqueous salicylic acid solution produces a blue-violet color. If the solution be very dilute, the color will be more of a red-violet. Excess of the reagent, or free hydrochloric acid, diminishes or entirely destroys the sensitiveness of the test. Hydrochloric acid changes the violet color to yellow.

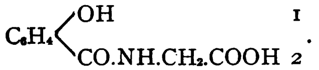
2. **Millon's Test.**—If an aqueous salicylic acid solution be warmed with Millon's reagent, a deep red color will appear.

3. **Bromine Water Test.**—This reagent produces a yellowish-white precipitate even with very dilute salicylic acid solutions.

4. **Melting-Point Test.**—If the quantity of salicylic acid be not too small, dissolve the ether residue in the smallest possible quantity of hot water, cool the solution and determine the melting point of the crystals.

Separation of Salicylic Acid from Simple Phenols.—When phenols like carbolic acid or the cresols are present, the above tests from 1 to 3 prove nothing as far as salicylic acid is concerned. If these compounds be present, add sufficient sodium carbonate solution to render the ether residue alkaline and extract the solution with ether. This solvent will take up the phenols and salicylic acid will remain in the water as the sodium salt. Withdraw the aqueous solution from the separating funnel, acidify and extract salicylic acid with ether.

Detection of Salicylic Acid in Urine.[1]—Salicylic acid forms a conjugate with glycocoll and is changed in the human body, in part at least, into salicyluric acid,



$$C_6H_4\left\langle\begin{array}{ll}OH & 1\\ CO.NH.CH_2.COOH & 2\end{array}\right.$$

This is eliminated in urine with unaltered salicylic acid. Such urine gives a violet color with ferric chloride solution.

Salicylic acid behaves in the human body like benzoic acid, which also enters into synthesis with glycocoll and is eliminated in urine as hippuric acid.

To isolate salicylic and salicyluric acids, acidify 200 to 300 c.c. of urine with hydrochloric acid and repeatedly extract with ether. Remove the ether from the aqueous solution in a separating funnel and shake vigorously with excess of sodium carbonate solution. Withdraw the aqueous solution, which is alkaline, acidify with dilute hydrochloric acid and extract with ether. A mixture of the two acids will be obtained by evaporating the ether. Purify the residue by recrystallization from water, using animal charcoal to remove color.

Salicyluric acid, like salicylic acid, gives a violet color with ferric chloride solution. Salicyluric acid, heated for 30 minutes under a reflux cooler with concentrated hydrochloric acid, gives glycocoll and salicylic acid.

To detect salicylic acid in wine, beer, fruits, milk and other similar materials, acidify 100 to 200 c.c. of liquid with dilute sulphuric acid and then extract with a mixture of equal parts of ether and petroleum ether. Test the residue, left by evaporating the solvent, for salicylic acid with ferric chloride solution.

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[1] To study the behavior of salicylic acid in this connection, take 1 to 1.5 grams of sodium salicylate at night and examine, as described above, the urine passed in the next 12 to 24 hours.

B. Extraction of the Alkaline Solution with Ether.

(Most of the alkaloids will appear here.)

Add enough sodium hydroxide solution to the acid, aqueous solution separated from ether to make it strongly alkaline. The alkali will liberate alkaloids from their salts and combine with morphine and apomorphine, should they be present. Thoroughly extract this alkaline solution with about the same quantity of ether. This solvent will dissolve all alkaloids except morphine, apomorphine and narceïne. Separate the ether from the aqueous solution and again extract with a fresh quantity of ether. In certain cases 3 or 4 such extractions may be required. Pour the ether extracts into a dry flask, stopper loosely and set aside for 1 or 2 hours. A few drops of water will always settle to the bottom of the flask. Carefully decant the ether and pour through a dry filter. Evaporate the filtrate with gentle heat in a glass dish (8 to 10 cm. in diameter). If small globules having a strong odor appear, the residue must be examined for coniïne and nicotine. If there be no trace of these volatile alkaloids, gently heat the residue upon the water-bath to expel completely water left by evaporation of the ether. Remove the dish from the water-bath as soon as this has been accomplished. It is not advisable to heat the residue too long. This residue, obtained by extracting the alkaline solution with ether, may contain any alkaloid except morphine, apomorphine and narceïne. It should be examined for

Coniïne
Nicotine
Aniline
Veratrine
Atropine (Hyoscyamine)
Scopolamine
Cocaïne
Physostigmine
Codeïne
Narcotine
Strychnine
Brucine
Quinine
Caffeïne
Antipyrine
Pyramidone.

First, note the general appearance of the residue and then examine it with the microscope. Taste it cautiously. Certain alkaloids may be recognized beforehand by this test. Special tests should then be made at once. The various alkaloids appear in the residue as follows:

Strychnine: Fine needles having an exceedingly bitter taste.

Brucine: Usually a white, amorphous powder having a very bitter taste.

Veratrine: Usually an amorphous powder having a sharp, burning taste.

Atropine and Quinine: A varnish which is resinous and sticky. Rarely crystalline or in the form of a powder.

Codeïne: A thick, viscous syrup which after a time becomes solid, especially if stirred with a glass rod, and frequently crystalline.

Caffeïne: Long, silky needles having a faintly bitter taste. These are frequently concentrically arranged.

Antipyrine: A syrup which gradually becomes crystalline, especially if stirred. It has a mild, bitter taste and dissolves very easily in water.[1]

Pyramidone: Usually as fine needles which have a faintly bitter taste. It is easily soluble in water.

Frequently ether leaves only a slight, tasteless residue. In that case alkaloids are absent. Such residues often consist of fat, resinous matter, or traces of nitrogenous substances (Peptones and their cleavage products? Creatinine?). Parts of a cadaver, even when quite fresh, usually give small residues at this point. Alkaloids may be absent and every step in the process may have been performed with the greatest care. To be absolutely certain that alkaloids are absent, dissolve a portion of the residue in water containing a drop of dilute hydrochloric acid. Filter, if necessary, and distribute this solution upon several watch-glasses. Make tests with the general, alkaloidal reagents, such as:

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[1] Most of the alkaloids are only slightly soluble in cold water. Some cannot be detected satisfactorily by purely chemical means. Others have no characteristic tests. Such substances should not be selected for laboratory practice. They may cause beginners to think that the experienced toxicologist relies upon similar uncertain methods when he seeks to identify an alkaloid in an actual analysis. I would place in this class alkaloids like nicotine, cocaïne, pilocarpine, scopolamine and physostigmine.

Mercuric Chloride
Iodine-Potassium Iodide
Potassium Mercuric Iodide
Potassium Bismuthous Iodide
Picric Acid
Tannic Acid
Phospho-Molybdic Acid
Phospho-Tungstic Acid.

If these reagents fail to give distinct and characteristic precipitates, alkaloids are absent. It is advisable in every instance to make this preliminary test for alkaloids. Only a small portion of material is required, and these general reagents show even traces of alkaloids.

To exclude mistakes and oversights in toxicological analysis, dissolve the ether residue, should it be very small, in a few c.c. of very dilute hydrochloric acid (about 1 per cent. of HCl). Evaporate this solution upon the water-bath and dissolve the residue in a small quantity of water. Inject this solution by means of a hypodermic syringe into the subcutaneous lymph-sac on the back of a small but lively frog. If the frog show no sign of poisoning in the course of several hours, it is quite likely that the residue does not contain any very poisonous alkaloid.

In making special tests for alkaloids, distribute the residue upon several watch-glasses, using a platinum or nickel spatula, or a small penknife. Or dissolve the residue in a small quantity of hot alcohol, filter the solution, distribute it upon watch-glasses and evaporate at a gentle heat. R. Mauch[1] dissolves the residue in 75 per cent. aqueous chloral hydrate solution and uses this solution in testing for alkaloids. (The details of this method will be found on page 109 of the Appendix at the end of this chapter.)

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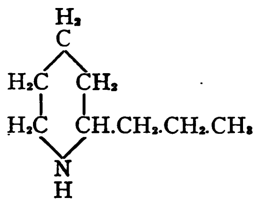
[1] Richard Mauch (Mittheilungen aus dem Institut des Herrn Prof. Dr. E. Schaer in Strassburg), "Festgabe des Deutschen Apotheker-Vereins," Strassburg, 1897.

Purification of the Alkaloidal Residue.—If the alkaloids be contaminated with greasy, resinous or fatty substances, many of the tests will either fail entirely or give uncertain results. When this is the case, the residue must be purified in one of two ways.

1. Thoroughly mix the residue with cold water containing hydrochloric acid. Filter to remove insoluble matter (fatty or resinous substances), add sodium hydroxide solution to the filtrate until alkaline and extract with ether. The alkaloids obtained by evaporating the solvent will usually be quite pure.

2. Or dissolve the residue in hot amyl alcohol, extract this solution with a few c.c. of very dilute sulphuric acid and withdraw the acid, aqueous solution from the separating funnel. Amyl alcohol will retain greasy and colored impurities, and the alkaloids will be in the aqueous solution as sulphates. Add sodium hydroxide solution in excess and extract with ether. This method of purifying the alkaloidal residue is especially recommended, when there is considerable coloring matter.

CONIÏNE.



Coniïne, or α-normal-propyl-piperidine (dextro-rotatory modification), occurs in spotted hemlock (Conium maculatum). It is a colorless, oily liquid which becomes resinous in contact with air. At the same time it acquires a yellow or brown color. It is slightly soluble in cold water but even less soluble in hot water. Coniïne is miscible with alcohol, ether, chloroform and benzene in all proportions. The unpleasant, narcotic odor of this alkaloid, sometimes said to resemble that of mouse urine, is far more intense than the odor of nicotine. General, alkaloidal reagents precipitate coniïne but the solution must not be too dilute. This is especially true of the chlorides of gold, platinum and mercury. Nicotine differs essentially from coniïne in giving precipitates with these reagents, even when its solutions are very dilute.

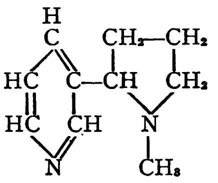
Coniïne may be recognized by the following tests:

1. **Solubility Test.**—Dissolve a drop of coniïne in just enough cold water to give a clear solution. Gently heat the solution and it will become milky, because coniïne is more easily soluble in cold water than in hot water. Aqueous coniïne solutions have an alkaline reaction.

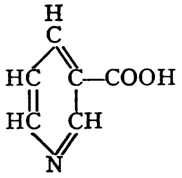
2. **Crystallization Test.**—Put a small quantity of coniïne upon a watch-glass, or glass slide, and add 1 or 2 drops of hydrochloric acid. Evaporate to dryness and coniïne hydrochloride will remain. Immediately after evaporation, examine this residue with a microscope magnifying about 200 times. The colorless or faintly yellow crystals are needle-like, or columnar, and are frequently grouped in star-shaped clusters. They show the play of color characteristic of doubly refractive substances.

3. **Tests with General Reagents.**—The general, alkaloidal reagents, iodine-potassium iodide, phospho-molybdic acid, potassium mercuric iodide and potassium bismuthous iodide are especially delicate with coniïne. It is hardly necessary to state, however, that these tests are not characteristic of this alkaloid.

Nicotine.



Nicotine is a colorless liquid, which soon turns yellow upon exposure to air and in time becomes resinous. It is miscible with water in all proportions, and freely soluble in alcohol, ether, amyl alcohol, benzene and petroleum ether. Chemically pure nicotine is said to be almost inodorous. The so-called tobacco odor is developed after the alkaloid has been for some time in contact with air. It has a sharp, burning taste. The free alkaloid is strongly laevo-rotatory but its salts are dextro-rotatory. Nicotine is a powerful poison and one of the most active alkaloids physiologically. Even in small quantity its vapor checks respiration. It is a di-acid base and its solutions are strongly alkaline. It forms salts with one or two equivalents of acid. Upon titration, with rosolic acid as indicator, nicotine behaves like a mon-acid base. Oxidized with chromic acid, potassium permanganate or nitric acid, nicotine is converted into nicotinic acid, or β-carboxy-pyridine. This shows that nicotine is a pyridine derivative having a side-chain in the β-position with respect to the pyridine nitrogen.



Phospho-tungstic acid is the most delicate, general alkaloidal reagent for nicotine. It produces distinct opalescence in a slightly acid solution of nicotine diluted 1 : 100,000. The limit of delicacy with phospho-molybdic acid is 1 : 40,000.

Nicotine may be recognized by the following tests:

1. **Crystallization Test.**—Evaporate nicotine with a few drops of concentrated hydrochloric acid. This will yield a yellow, varnish-like residue which microscopic examination will show to be entirely amorphous (Distinction between nicotine and coniïne). If kept for a long time in a

desiccator over sulphuric acid, it will become indistinctly crystalline.

2. **Roussin's Test.**—Dissolve a trace of nicotine in ether, using a dry test-tube. Add about the same volume of ether containing iodine to this solution. Stopper and set the test-tube aside. The mixture will become turbid and deposit a brownish-red resin which will gradually become crystalline. After some time, ruby-red needles with a dark blue reflex will crystallize from the ether. These are "Roussin's crystals." If nicotine be old or resinous, it will not as a rule give these crystals.

ANILINE.

Aniline, $C_6H_5.NH_2$, upon evaporation of the ether extract from the alkaline solution, will usually appear as reddish or brownish globules. Dissolve some of this residue in water and apply the aniline tests already described. A further test for aniline consists in mixing some of the residue with a few drops of concentrated sulphuric acid, and adding a few drops of potassium dichromate solution. If aniline be present, an evanescent blue color will appear.

VERATRINE.

The officinal veratrine, prepared from sabadilla-seeds (Sabadilla officinarum), is not an individual compound but an intimate mixture of several alkaloids, the separation of which is attended with considerable difficulty. The principal constituent of the officinal preparation is the crystalline compound, cevadine ($C_{32}H_{49}NO_9$), also called crystalline veratrine. It contains, moreover, amorphous veratrine, the two crystalline substances, sabadine and sabadinine, and finally sabadilline.

Veratrine appears as a white powder which is seen upon microscopic examination to be crystalline. It has a sharp, burning taste and the minutest quantity introduced into the nostrils excites protracted sneezing. It is almost insoluble in water; fairly soluble in ether, benzene, petroleum ether and amyl alcohol; and freely soluble in alcohol and chloroform. If the veratrine solution be faintly acid, ether will extract a very small quantity of the alkaloid. Under the same conditions, chloroform and amyl alcohol will extract much more. The alkaloid is usually deposited from ether as a white, amorphous powder. Phospho-molybdic acid, iodine-potassium iodide, tannic acid and potassium mercuric iodide give distinct precipitates with an aqueous veratrine solution containing hydrochloric acid and diluted 1 : 5000. Chlorides of gold and platinum and picric acid fail to show the alkaloid in this dilution.

Veratrine may be recognized by the following tests:

1. **Concentrated Sulphuric Acid Test.**—Pour a few drops of concentrated sulphuric acid upon a trace of veratrine. The alkaloid will have an intense yellow color and, if stirred, will give a solution of the same color. Gradually this color will change to orange, then to blood-red and finally to cherry-red. Gentle heating will hasten this color change and veratrine, dissolved in concentrated sulphuric acid, will give a beautiful cherry-red solution almost immediately. Froehde's reagent has the same action upon veratrine. Erdmann's reagent behaves similarly but the color change takes place more quickly.

2. **Concentrated Hydrochloric Acid Test.**—If a trace of veratrine be dissolved in 1 or 2 c.c. of cold, concentrated hydrochloric acid, the solution will be colorless. When this solution is heated 10 to 15 minutes in a boiling water-bath, a beautiful cherry-red color will appear. This color will last for a day and even 0.2 milligrams of veratrine will produce it. This is a very delicate test and is characteristic of veratrine. It is advisable to employ a test-tube in making this test.

3. **Concentrated Nitric Acid Test.**—Concentrated nitric acid dissolves veratrine with a yellow color.

4. **Weppen's Test.**—Thoroughly mix 1 part of veratrine with about 5 parts of finely powdered cane-sugar in a mortar. Add a few drops of concentrated sulphuric acid to some of the mixture upon a watch-glass. At first the mixture will have a yellow color which later, beginning at the edge, will change to grass-green and finally to blue. Breathing upon the mixture will cause the color to change more quickly. Too great an excess of cane-sugar must be avoided.

E. Laves[1] states that an aqueous furfurol solution may be substituted for cane-sugar in this test. Mix in a test-tube 3 or 4 drops of 1 per cent. aqueous furfurol solution with 1 c.c. of concentrated sulphuric acid. Add 3 to 5 drops of this solution to the substance to be tested so that it just touches the edge of the liquid. If veratrine be present, a dark streak will gradually run from the substance into the liquid. At the starting-point it will appear blue or blue-violet and farther away green. If substance and liquid be stirred with a glass rod, the liquid will become dark green. After some time, or more quickly when warmed, the color will become blue and finally violet.

5. **Vitali's Test.**—Dissolve veratrine in a few drops of fuming nitric acid, and evaporate the solution to dryness upon the water-bath. A yellowish residue will remain. If this be cooled and then moistened with an alcoholic potassium hydroxide solution, the color will change to red or red-violet, and stirring will produce a solution having the same color. Atropine gives the same test.

STRYCHNINE.

Strychnine, $C_{21}H_{22}N_2O_2$, crystallizes in colorless, shining prisms belonging to the rhombic system. It is soluble in water with very great difficulty and has an intensely bitter taste. Strychnine diluted with water 1 : 600,000 can be recognized distinctly by its bitter taste. The alkaloid is nearly insoluble in absolute alcohol and in absolute ether. It is more easily soluble in commercial ether and benzene. Chloroform is the best solvent of all. Tannic acid, picric acid, iodine-potassium iodide, potassium mercuric iodide and potassium bismuthous iodide are the most delicate, general alkaloidal reagents for strychnine. It is advisable to use solutions of strychnine salts for these tests. The ether residue may be dissolved in very dilute hydrochloric acid for this purpose.

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[1] *Pharmaceutische Zeitung,* 37, 338.

Concentrated sulphuric acid, as well as Erdmann's and Froehde's reagents, dissolves perfectly pure strychnine without color. Concentrated nitric acid dissolves strychnine with a yellowish color which later becomes dark yellow. If the solution of strychnine salt be not too dilute, potassium dichromate solution will give a yellow, crystalline precipitate of strychnine chromate consisting of fine needles. Potassium ferricyanide precipitates yellow, crystalline strychnine ferricyanide from solutions of strychnine salts.

Strychnine may be recognized by the following tests:

1. **Sulphuric Acid-Dichromate Test.**—Dissolve a very small quantity of strychnine in two or three drops of concentrated sulphuric acid upon a watch-glass. The solution should be colorless. Add a fragment of potassium dichromate and hold it firmly in one place upon the glass. Intense blue or blue-violet streaks will come from the potassium dichromate, if the watch-glass be moved up and down. If the entire mixture be stirred, the sulphuric acid will be colored a beautiful blue or blue-violet. Or the test may be made by scattering upon the surface of the solution of strychnine in concentrated sulphuric acid a few particles of coarsely powdered potassium dichromate and mixing well with a

glass rod. The blue color is not permanent. It soon changes to red and finally to yellow.

Strychnine chromate and ferricyanide give this test especially well. To prepare the former salt, pour a very dilute solution of potassium dichromate upon strychnine in a watch-glass. When the two substances have been in contact for some time, pour the remaining liquid from the strychnine chromate. Wash the precipitate once with a small quantity of water. Put some strychnine chromate upon the end of a glass rod and draw it through a few drops of concentrated sulphuric acid upon a watch-glass. This will produce beautiful violet and blue streaks in the acid.

2. **Physiological Test.**—Dissolve the ether residue in a small quantity of very dilute hydrochloric acid. Evaporate this solution to dryness upon the water-bath. Dissolve the residue in pure water (about 1 c.c.) and inject this solution into the lymph-sac on the back of a small but lively frog. Evidence of poisoning will appear in 5 to 30 minutes, depending upon the quantity of strychnine. It is best to keep the experimental frog in a large beaker which is loosely covered. Strychnine does not increase reflex irritability for all kinds of stimuli but only for tactile, optical and especially for acoustic stimuli. (Kobert, Intoxikationen.) When the dose of strychnine is sufficiently large, each kind of stimulus mentioned will produce spasms like those caused by tetanus.

BRUCINE.

Brucine, $C_{23}H_{26}N_2O_4$, crystallizes from dilute alcohol in colorless, transparent plates. It is usually obtained in an amorphous form by evaporating the ether solution. It dissolves easily in alcohol and chloroform and less easily in absolute ether. Brucine, however, is more soluble in ether than strychnine. Solutions of this alkaloid have a very bitter taste and an alkaline reaction. Iodine-potassium iodide, potassium mercuric iodide, potassium bismuthous iodide, tannic and phospho-molybdic acids are the most delicate, general alkaloidal reagents for brucine.

Brucine appears to be a di-methoxyl compound of strychnine. The difference in the percentage composition of the two alkaloids and the evidence of the presence of two methoxyl-groups in brucine seem to be in favor of this view. The presence of such a methoxyl-group (— OCH_3) has not been detected in the strychnine molecule.

Brucine may be recognized by the following tests:

1. **Nitric Acid-Stannous Chloride Test.**—Concentrated nitric acid dissolves brucine and its salts with a blood-red color. This color, however, is slightly stable and soon changes to yellowish-red and finally, especially with heat, to yellow. Add a few drops of freshly prepared, dilute stannous chloride solution to this yellowish-red or yellow solution. An intense violet color will appear. Heat usually changes this violet color again to yellowish-red, but addition of a few more drops of stannous chloride solution will cause the violet color to reappear. The smaller the quantity of nitric acid, the more chance there is that this test will give a good result. A colorless ammonium sulphide solution may be substituted for stannous chloride.

2. **R. Mauch's[1] Modification of Nitric Acid-Stannous Chloride Test.**—An excellent result can be obtained with this test in the following manner. Dissolve brucine in 60 percent. aqueous chloral hydrate solution and put about 0.5 c.c. of this solution into a test tube. Add a very small quantity of dilute nitric acid and thoroughly mix the two solutions. Add this mixture to three times its volume of concentrated sulphuric acid so that the former is on the surface. A yellowish-red to deep red zone, depending upon the quantity of brucine, will appear immediately. When the

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[1] *Loc. cit.*, see page 70.

upper layer becomes yellow, introduce by a pipette a little stannous chloride solution as a top layer. Prepare stannous chloride solution by dissolving 1 part of stannous chloride in 9 parts of hydrochloric acid having a specific gravity of 1.12 (about 24 per cent. HCl). A brilliant, intensely violet zone will appear between the two upper layers. The intensity of this color will gradually increase, especially if the test-tube be gently moved to and fro.

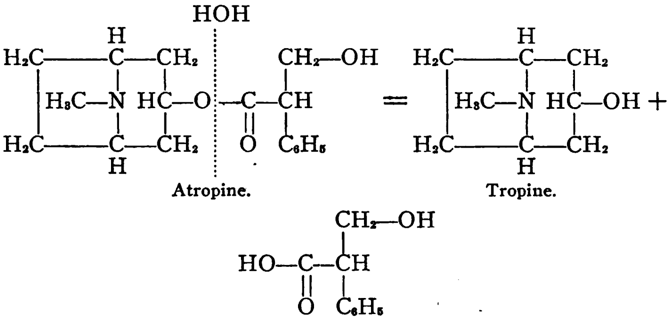
3. **Nitric Acid-Ammonia Test.**—Add a few drops of concentrated nitric acid to brucine upon a watch-glass and evaporate the solution to dryness. An orange-red to brownish residue will remain. Ammonia gas will change this color to grass-green. Dissolved in 2 or 3 c.c. of hydrogen sulphide water, it will give a violet color. This is a very delicate test but not sharp in presence of much strychnine. To expose the residue to ammonia gas, place the watch-glass upon another which contains a few drops of ammonium hydroxide solution.

Many alkaloids and other substances give yellow residues when evaporated with concentrated nitric acid, for example, [illegible]gdalin, strychnine, cotoïne, emodine, narcotine, physostigmine, salicin.

Detection of Strychnine and Brucine when Together.—Concentrated sulphuric acid dissolves a mixture of these two alkaloids without color. Addition of a trace of nitric acid or a fragment of potassium nitrate will produce the red color characteristic of brucine. This color will soon change to yellow. Then add a small crystal of potassium dichromate. The liquid, when stirred, will become blue or blue-violet, if strychnine be present. (See strychnine.)

ATROPINE.

Atropine, $C_{17}H_{23}NO_3$, crystallizes in shining, colorless, pointed needles. It is soluble in about 600 parts of water, 50 parts of ether and 3.5 parts of chloroform. A hot, aqueous solution of the free alkaloid is strongly alkaline and has an unpleasant, persistently bitter taste. Concentrated sulphuric acid dissolves atropine without color. The solution, however begins to darken, if heated. Iodine-potassium iodide, potassium mercuric iodide, potassium bismuthous iodide, phospho-molybdic and phospho-tungstic acids are especially delicate reagents for atropine. The other general reagents produce precipitates only with concentrated solutions. Heated with barium hydroxide solution, atropine is decomposed into tropine, which is an alcohol, and tropic acid. Atropine is, therefore, the tropyl ester of tropic acid.



The constitution of tropine and tropic acid has been definitely determined by synthesis and by studying their decomposition products. In this way the structure of atropine has been ascertained. Hyoscyamine is a stereoisomer of atropine. It is converted into atropine, when heated to 110° out of contact with air, or when allowed to stand dissolved in alcohol containing a few drops of alkali. This change may occur even when hyoscyamine is kept for a long time. Atropine is probably the racemic form, and laevo-rotatory hyoscyamine is the laevo-modification of this isomeric base.

Atropine may be recognized by the following tests:

1. **Vitali's Test.**—This is an excellent test for atropine. Dissolve the alkaloid in a few drops of fuming nitric acid, and evaporate the solution in a porcelain dish to dryness upon the water-bath. The residue will be nearly colorless. Moisten the residue when cold with a few drops of a solution of potassium hydroxide in absolute alcohol. A beautiful violet color will appear and soon change to cherry-red. Veratrine behaves in a similar manner. (See page 76.) Consequently, Vitali's test is characteristic of atropine, only when veratrine is absent. Atropine can be recognized with certainty in presence of veratrine by its characteristic physiological action.

2. **Odor Test.**—Heat a small quantity of atropine in a dry test-tube until a white vapor appears. An agreeable odor will arise at the same time. Then add about 1 c.c. of concentrated sulphuric acid, and heat until the acid begins to darken. Dilute at once with about 2 c.c. of water. During the foaming there will be an intense, sweetish odor like that of honey. By means of this test, which was formerly the only method of identifying atropine, 0.01 grams of the alkaloid can be detected.

3. **Physiological Test.**—Atropine acts in a very characteristic manner upon the pupil of the eye, and this behavior can be employed as a test. One drop of an atropine solution diluted 1 : 130,000 will produce a noticeable enlargement of the pupil. Dissolve a small portion of the ether residue in 4 or 5 drops of very dilute sulphuric acid, and introduce a drop of this solution into one eye. The enlargement of the pupil often persists for several hours. The utmost care should be taken in performing this test.

Homatropine, the tropyl ester of phenylglycolic or mandelic acid, has the structure $C_8H_{14}NO - CO.CH(OH).C_6H_5$.[1] This substance is used in medicine as a substitute for atropine for two reasons. First, because its action upon the pupil is nearly as vigorous as that of the natural alkaloid. And further, because its effect does not last more than 12 to 24 hours, whereas that caused by atropine often continues for 8 days. Moreover, its toxicity is less. Homatropine hydrochloride is obtained by heating a mixture of tropine, mandelic acid and hydrochloric acid. In this reaction the mineral acid is used as a means of abstracting a molecule of water. Homatropine is a strong base and forms neutral salts with acids. It melts at 92 to 96°; hyoscyamine at 108°; and atropine at 115.5°. Homatropine gives Vitali's test.

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[1] $C_8H_{14}NO$ is the tropyl radical.

PHYSOSTIGMINE.

Physostigmine, $C_{15}H_{21}N_3O_2$, also called eserine, is the alkaloid of Calabar bean, the seed of Physostigma venenosum. It is a strong, tertiary, mon-acid base. It is laevorotatory and a very powerful poison. The pure alkaloid appears in colorless crystals which are soluble with difficulty in water but easily soluble in ether, chloroform and alcohol. It is almost tasteless. Eserine solutions are very easily decomposed. They turn red, especially when exposed to the action of air and light. The alkalies, ammonia and the alkaline carbonates precipitate the alkaloid from solutions of its salts, and at the same time they produce a marked red color in the solution.

Physostigmine may be recognized by the following tests:

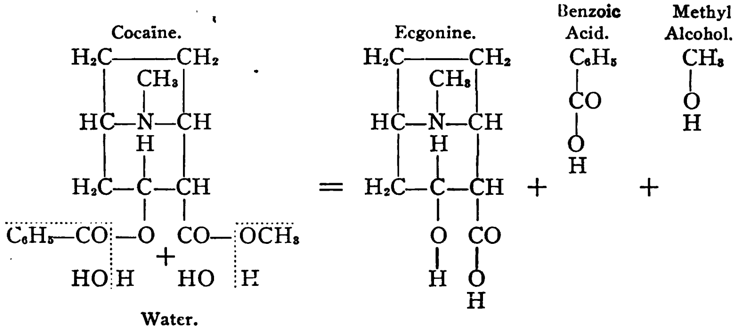
1. **Ammonia Test.**—Hot ammonia added to an eserine salt will produce a yellowish-red solution. If this be evaporated upon the water-bath, a blue or blue-green residue will remain. This will dissolve in alcohol with a blue color. Excess of acetic acid, added to this solution, will change the color to red. The solution is also strongly fluorescent, being violet by transmitted and red by reflected light. A drop of concentrated sulphuric acid, added to the blue residue from evaporation with ammonia, will give a green solution. The green color diluted with alcohol will change to red. If the alcohol be evaporated, the green color will reappear.

2. Physiological Test.—Physostigmine produces marked contraction of the pupil. This action is very characteristic of the alkaloid and will show even 0. 1 milligrams.

COCAÏNE.

Cocaïne, $C_{17}H_{21}NO_4$, crystallizes from alcohol in large, colorless prisms which melt at 98°. It has a bitterish taste and, placed upon the tongue, causes temporary, local anaesthesia. The alkaloid is only slightly soluble in water (1 : 700), but easily soluble in alcohol, ether, chloroform, benzene and acetic ether. Its solutions are strongly alkaline and laevo-rotatory. Dilute acids easily dissolve cocaïne and in most cases form readily crystallizable salts. The fixed alkalies, ammonia and alkaline carbonates precipitate the free base from solutions of its salts.

Cocaïne is a tertiary base which contains one methoxyl-group and one methyl group attached to a nitrogen atom. Moreover, it is an ester. Cocaïne boiled with water is decomposed into benzoyl ecgonine and methyl alcohol. If a mineral acid, or a solution of barium hydroxide or of an alkali, be substituted for water, benzoyl ecgonine also will be decomposed. Cocaïne, therefore, yields ecgonine, benzoic acid and methyl alcohol.



Nearly all the general, alkaloidal reagents give precipitates with solutions of cocaïne salts. Iodine-potassium iodide, potassium mercuric iodide, phospho-molybdic, phospho-tungstic and picric acids are especially delicate.

Cocaïne may be recognized by the following tests:

1. **Concentrated Acid Test.**—Concentrated sulphuric and nitric acids dissolve cocaïne without color.

2. **Precipitation Test.**—If 1 or 2 drops of potassium hydroxide solution be added to an aqueous solution of a cocaïne salt not too dilute, the solution will become milky. First, resinous globules and later fine crystalline needles of the free base, cocaïne (melting point 98°), separate from solution. In applying this test to the ether residue, dissolve a considerable quantity in a few drops of dilute hydrochloric acid and add potassium hydroxide solution drop by drop until alkaline. This test is not characteristic of cocaïne (except the melting-point which, however, requires considerable pure material) because most of the alkaloids are precipitated by potassium hydroxide solution in much the same way.

3. **Potassium Permanganate Test.**—Add potassium permanganate solution (1 : 100) drop by drop to a concentrated, aqueous solution of a cocaïne salt. This reagent will give a violet, crystalline precipitate of cocaïne permanganate. In applying this test to the ether residue, dissolve a considerable quantity in 2 drops of dilute hydrochloric acid and add permanganate solution drop by drop. This is a delicate test for cocaïne.

4. **Chromic Acid Test.**—Add a few drops of 5 per cent. chromic acid solution to a solution of a cocaïne salt. Each drop will produce a precipitate which will immediately disappear, if the solution be shaken. Then add to the clear solution about 1 c.c. of concentrated hydrochloric acid which will produce a more or less crystalline precipitate of orange-yellow cocaïne chromate.

5. Detection of Benzoyl Group in Cocaïne.—This test requires at least 0.2 grams of cocaïne. First, digest the cocaïne a few minutes with 2 c.c. of concentrated sulphuric acid upon a boiling water-bath. Cool and dilute with a small quantity of water, all the while keeping the mixture cold. A white, crystalline precipitate of benzoic acid will appear. Collect and dry this precipitate upon a filter. Benzoic acid can be recognized by subliming the precipitate, or, if the quantity be sufficient, by determining the melting-point (120°).

Benzoic acid can also be extracted with ether. Mix the residue, obtained by evaporating the ether, with 1 c.c. of absolute alcohol and the same quantity of concentrated sulphuric acid. The characteristic odor of ethyl benzoate will be produced.

6. Physiological Test.—Cocaïne can be detected with certainty by this test which depends upon the fact that this alkaloid causes a peculiar, temporary, local anaesthesia. Dissolve the material (the residue from the ether extract) in a few drops of dilute hydrochloric acid and evaporate the solution to dryness upon the water-bath. Dissolve the residue in a small quantity of pure water, and apply this solution to the tongue.

7. M. Goeldner's[1] Test.—Mix about 0.01 grams of pure resorcinol in a small dish with 6 or 7 drops of pure, concentrated sulphuric acid. Add about 0.02 grams of cocaïne hydrochloride to this solution which usually has a faint, yellowish color. There is a vigorous reaction, during which the liquid acquires a beautiful blue color like that of the cornflower. The intensity of this color gradually increases. Sodium hydroxide solution will change the blue color to light pink.

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[1] *Pharmaceutische Zeitschrift für Russland,* 28, 489, and *Zeitschrift für analytische Chemie,* 40, 820 (1901).

CODEÏNE.

Codeïne, $C_{17}H_{18}(CH_3)NO_3$, the methyl ether of morphine, crystallizes from water, or from ether containing water, in colorless, transparent octahedrons which are often very large. These crystals are quite easily soluble in water. One part of the free base is soluble at 15° in 80 parts of water and at 100° in 15 parts. Codeïne differs from most of the other alkaloids, morphine, for example, by its relatively high solubility in water. Alcohol, ether, amyl alcohol, chloroform and benzene also dissolve codeïne freely. It is, however, practically insoluble in petroleum ether. Aqueous codeïne solutions are strongly alkaline and bitter. Pure codeïne does not reduce iodic acid, nor does it immediately produce a blue color or a blue precipitate in a mixture of potassium ferricyanide and ferric chloride solutions. (Difference between morphine and codeïne.) Phospho-molybdic acid, iodine-potassium iodide, potassium bismuthous iodide and potassium mercuric iodide give precipitates even with very dilute codeïne solutions.

Codeïne may be recognized by the following tests:

1. **Sulphuric Acid Test.**—Concentrated sulphuric acid dissolves codeïne without color. After long contact or application of gentle heat, the solution will have a reddish to violet-red color. Tambach and Henke[1] state that a deep red solution is obtained by adding powdered codeïne to sulphuric acid at ordinary temperatures, as long as it continues to be dissolved.

2. **Nitric Acid Test.**—Cold nitric acid (25 per cent.) will convert codeïne into nitro-codeïne. At the same time the acid will dissolve the alkaloid with a yellow color which will soon change to red. This test is not characteristic of codeïne.

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[1] *Pharmaceutische Zentralhalle,* 38, 159.

3. **Oxidation Test.**—Pour a few drops of concentrated sulphuric acid upon a small quantity of codeïne in a watch-glass. Then add finely powdered potassium arsenate (KH_2AsO_4), and gently warm over a small flame. The acid will have a deep blue or blue-violet color. Excess of potassium arsenate does not affect the test. If water or sodium hydroxide solution be added, the blue color will change to orange yellow.

A trace of ferric chloride solution may be used instead of potassium arsenate. Sulphuric acid, containing 1 drop of ferric chloride solution to 10 c.c. of acid, is prescribed by the "German Pharmacopoeia," IV edition, for detecting the alkaloid in codeïne phosphate.

4. **Test With Froehde's Reagent.**—This reagent dissolves codeïne with a yellowish color which soon changes to green and later to blue. Gentle warming will hasten this change of color. R. Mauch's method may be used in this test by warming 2 or 3 drops of a chloral hydrate solution of codeïne with about 10 drops of Froehde's reagent. An intense blue color will appear.

5. **Furfurol Test.**[1]—Dissolve codeïne in a few drops of concentrated sulphuric acid and warm very gently with a drop of cane-sugar solution (1 : 10). This will produce a purple-red color. Excess of sugar interferes with this test.

This test may also be made by mixing a drop of sugar solution with codeïne, dissolved in about 5 drops of 50 to 60 per cent. aqueous chloral hydrate solution, and then adding concentrated sulphuric acid as an under layer. A carmine-red ring will appear at the zone of contact. The color is quite permanent and increases in intensity upon standing. If the sulphuric acid and chloral hydrate solution be thoroughly mixed, the entire liquid will be red. After a time the shade of color will be more of a red-brown.

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[1] This test depends upon furfurol formed by the action of concentrated sulphuric acid upon cane-sugar. A very dilute, aqueous furfurol solution (1 : 1000) may be substituted for cane-sugar. Excess of furfurol, unlike cane-sugar, does not interfere with the test. Tr.

6. Pellagri's Test.—Both codeïne and morphine give this test. Dissolve codeïne in concentrated hydrochloric acid and add at the same time 3 or 4 drops of concentrated sulphuric acid. Expel hydrochloric acid upon the water-bath and afterwards heat the residue about 15 minutes. Dissolve the dirty red or violet residue in 2 or 3 c.c. of water, add a few drops of hydrochloric acid and neutralize with acid sodium carbonate. Then add an alcoholic solution of iodine drop by drop (2 to 4 drops) and shake thoroughly for several minutes. An emerald-green solution indicates codeïne. Extract the green solution with ether. The color of the ether will be red, whereas that of the aqueous solution will remain green. This is a test for apomorphine (see page 97) formed from codeïne by the mineral acid.

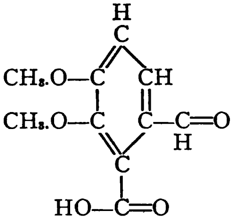
$$\underset{\text{Codeïne.}}{C_{17}H_{18}(CH_3)NO_3} + HCl = \underset{\text{Apomorphine.}}{C_{17}H_{17}NO_2} + CH_3.Cl + H_2O.$$

NARCOTINE.

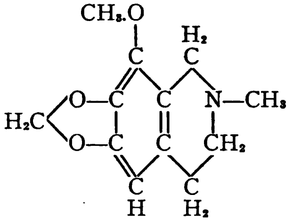
Narcotine, $C_{22}H_{23}NO_7$, crystallizes in shining prisms or flat needles. This alkaloid differs essentially from the other constituents of opium in being neither alkaline nor bitter. Next to morphine it is the principal alkaloid in opium. But it is not nearly as poisonous as morphine. Narcotine is nearly insoluble in cold water but quite readily soluble in ether, alcohol and chloroform. Its salts are unstable and do not crystallize. Because of its feebly basic character, narcotine can be completely extracted from a tartaric acid solution by chloroform. This makes it easy to separate narcotine from the opium alkaloids, especially from codeïne, and also from other alkaloids. Chloroform and ether usually deposit narcotine as a faintly colored varnish. This residue solidifies upon long standing and forms a mass of radiating crystals. The general, alkaloidal reagents, iodine-potassium iodide, phospho-molybdic acid, potassium mercuric iodide and potassium bismuthous iodide, precipitate narcotine from very dilute hydrochloric or sulphuric acid solution. Narcotine is a weak, tertiary base. If it be heated to 140° with water, sulphuric acid or barium hydroxide solution, it will yield opianic acid, a nitrogen-free compound, and the base, hydrocotarnine.

$$\underset{\text{Narcotine.}}{C_{22}H_{23}NO_7} + H_2O = \underset{\text{Opianic Acid.}}{C_{10}H_{10}O_5} + \underset{\text{Hydrocotarnine.}}{C_{12}H_{15}NO_3.}$$

The constitution of these cleavage products of narcotine has been definitely determined.



Opianic Acid.



Hydrocotarnine.

Narcotine may be recognized by the following tests:

1. **Test with Froehde's Reagent.**—This reagent dissolves narcotine with a greenish color. If concentrated Froehde's reagent be used, the green color will change to a beautiful cherry-red, especially upon application of gentle heat. This color lasts quite a long time.

2. **Sulphuric Acid Test.**—Dissolve narcotine in dilute sulphuric acid and evaporate the solution in a dish upon the water-bath. At first the color is orange, but it soon begins at the edge to change to bluish-violet. Finally, if the solution be heated upon an asbestos plate, until sulphuric acid fumes begin to appear, it will have a dirty reddish-violet

color. If heating be stopped before the violet color appears, the solution as it cools will gradually assume a cherry-red color.

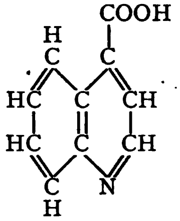
3. **Concentrated Sulphuric Acid Test.**—If the yellow solution of narcotine in concentrated sulphuric acid be carefully heated, the same color changes mentioned in the second test will be observed.

4. **Sulphuric-Nitric Acid Test.**—Dissolve narcotine in cold concentrated sulphuric acid and mix a trace of nitric acid with this solution after 1 or 2 hours. A red color will appear and gradually become more and more pronounced. Narcotine dissolved in Erdmann's reagent will give the same result.

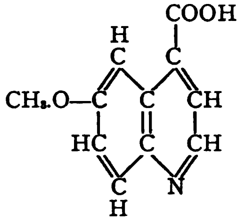
QUININE.

Quinine, $C_{20}H_{24}N_2O_2$, is precipitated amorphous and free from water of crystallization when alkalies are added to solutions of its salts. But it gradually becomes crystalline and combines with three molecules of water of crystallization. Under certain conditions quinine also forms other hydrates. When sodium carbonate solution is added to a quinine salt dissolved in hot water, the anhydrous alkaloid will separate as small needles. The anhydrous alkaloid melts at 173°; the trihydrate at 57°. Quinine is usually deposited from ether as a resinous or varnish-like mass. It is soluble at 15° in about 2000 parts of water and at 100° in 700 parts. It is freely soluble in alcohol, ether and chloroform. A solution of quinine in sulphuric, acetic or tartaric acid has a beautiful, blue fluorescence. In the case of sulphuric acid this fluorescence can be distinctly recognized in a dilution of 1 : 100,000. Solutions of the alkaloid in hydrochloric and hydrobromic acids are not fluorescent. In fact, the fluorescence of a quinine solution will cease upon addition of either acid.

Cinchonine and quinine, oxidized with chromic acid, yield respectively among other products cinchoninic and quininic acids. The former is quinoline-γ-carboxylic acid and the latter methoxyquinoline-γ-carboxylic acid.



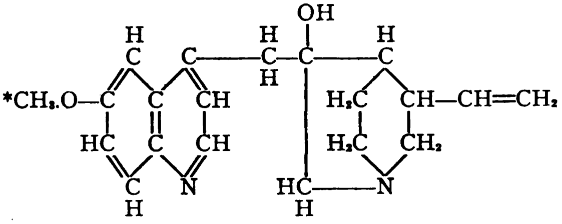
Cinchoninic Acid.



Quininic Acid.

This shows that a quinoline ring is present in the molecule of these two cinchona alkaloids (the so-called "quinoline half" of the cinchona alkaloids).

W. Königs[1] has proposed the following structural formula for quinine:



Quinine.

Quinine, therefore, is a di-acid, di-tertiary base which contains one hydroxyl- and one methoxyl-group. In cinchonine the methoxyl-group marked with an asterisk is replaced by an atom of hydrogen. Consequently, quinine is a methoxycinchonine.

Quinine may be recognized by the following tests:

1. **Fluorescence Test.**—Dissolve the residue from the ether extraction of the alkaline solution in a small quantity

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[1] *Journal für praktische Chemie* (2), 61, 1–46 (1899).

of dilute sulphuric acid. This solution will show a blue fluorescence, when quinine is present.

2. **Thalleïoquin Test.**—Dissolve quinine in a few drops of very dilute acetic acid, and add 5 to 10 drops of saturated chlorine water. The solution will either be colorless or have a faint, blue fluorescence. Addition of ammonium hydroxide solution in excess will produce an emerald-green color. A solution containing considerable quinine will give a green precipitate. This precipitate, thalleïoquin, is always an amorphous, non-crystalline substance, the composition of which has not been determined. It is soluble in alcohol and chloroform but not in ether.

E. Polacci[1] recommends the following procedure for the thalleïoquin test. Gradually heat quinine (about 0.01 grams) to boiling with a small quantity of lead peroxide, 2 or 3 c.c. of water and 2 drops of dilute sulphuric acid. Let the solution settle and either decant or filter. Finally, carefully add 5 or 6 drops of ammonium hydroxide solution as a top layer. A beautiful green ring will appear at the zone of contact.

Interferences with the Thalleïoquin Test.—Antipyrine is detrimental to this quinine test. Chlorine water and ammonium hydroxide solution will give a very beautiful red color instead of green with mixtures of 1 per cent. solutions of antipyrine and quinine. This interference does not cease until these two substances are in the proportion of 0.25 parts of antipyrine to 5 parts of quinine. Caffeïne also interferes with the thalleïoquin test, when the alkaloids are mixed in the proportion of 2 parts of quinine to 3 parts of caffeïne. Other compounds like urea prevent the appearance of this color, whereas morphine, pilocarpine, cocaïne, atropine, codeïne, strychnine, carbolic acid and chloral hydrate have no effect upon the thalleïoquin test.

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[1] *Gazetta chimica italiana,* 28, 391.

3. **Herapathite Test.**—Mix 30 drops of acetic acid, 20 drops of absolute alcohol and 1 drop of dilute sulphuric acid. Add 20 drops of this mixture to 0.01 gram of quinine and heat to boiling. Finally add 1 drop of an alcoholic solution of iodine (1 : 100). When the solution has stood for some time, green leaflets with a metallic lustre will form. This is an iodine compound of quinine called "Herapathite." This substance can be recrystallized from boiling alcohol. Because of its slight solubility in water, herapathite can be used in the quantitative determination of quinine, especially in the quantitative separation of quinine from the other cinchona alkaloids which give freely soluble iodine compounds.

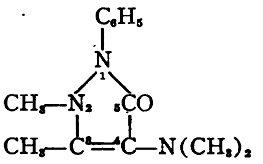
CAFFEÏNE.

Since caffeïne is a weak base, ether will extract only a small quantity of the alkaloid from the tartaric acid solution. The greater part will be found in the ether extract of the alkaline solution. Ether usually deposits caffeïne in white, shining needles arranged in clusters. It may be recognized [illegible]he tests already given. (See page 65.)

ANTIPYRINE.

Most of the antipyrine, as has been stated elsewhere, is obtained by extracting the alkaline solution with ether. It is usually much purer when extracted from the alkaline solution and frequently appears in crystalline leaflets. Antipyrine differs from most of the true alkaloids in having only a faint, bitter taste and in being freely soluble in water. To identify antipyrine, extract the ether residue with 10 to 20 drops of cold water and divide the solution into two equal parts. Test one portion with ferric chloride solution and the other with fuming nitric acid.

PYRAMIDONE.



Pyramidone, or 4-dimethyl-amido-antipyrine, has been extensively used in medicine of late as an antipyretic and anodyne. It is a white, crystalline powder, nearly tasteless and readily soluble in water. It melts at 108°. Its aqueous solution has a neutral reaction. Ether removes only traces of pyramidone from tartaric acid solution, but extracts it easily and completely from alkaline solution. Ether usually deposits this substance in fine needles. Pyramidone is also freely soluble in alcohol, chloroform and benzene. It is a strong reducing agent and in this respect differs from antipyrine. For example, pyramidone will reduce gold chloride even in the cold, whereas antipyrine and tolypyrine require heat.

After administration of pyramidone, human urine usually has a light, purple-red color and a neutral or faintly acid reaction. Upon standing for some time, the urine deposits a sediment consisting of small, red needles which are soluble in ether and chloroform but especially in acetic ether. Jaffé[1] found this substance to be rubazonic acid, a pyrazolone derivative.

Pyramidone may be recognized by the following tests:

1. **Ferric Chloride Test.**—Ferric chloride solution added to pyramidone produces a blue violet color which soon changes to reddish-violet and then disappears.

2. **Fuming Nitric Acid Test.**—A few drops of fuming nitric acid, added to a solution containing pyramidone, give a blue or blue-violet color.

3. **Bromine Water Test.**—This reagent imparts a grayish color to pyramidone solutions. But with concentrated solutions it produces an inky color.

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[1] *Berichte der Deutschen chemischen Gesellschaft,* 34. 2737 (1901).

C. EXTRACTION OF THE AMMONIACAL SOLUTION WITH ETHER AND CHLOROFORM.

(*a*) The ether extract will contain apomorphine and traces of morphine[1].

(*b*) The chloroform extract will contain morphine and narceïne. (It may also contain antipyrine and caffeïne.[2])

The aqueous, alkaline solution separated from ether may further contain apomorphine, morphine and narceïne.

Apomorphine may be recognized by the beautiful, green color of the acid, aqueous solution. Excess of sodium hydroxide solution causes oxidation, especially if the solution be exposed for any length of time to air, and gradually changes the color to deep purple-red. Moreover, the ether extracts, both of the acid and alkaline solutions, are red or violet-red when apomorphine is present. Solutions, examined by the Stas-Otto method and having these characteristics, must be tested for apomorphine. That is to say, the liquid made alkaline with ammonium hydroxide solution must be extracted with ether. But when a solution does not show these characteristics, this extraction may be omitted. At this point in the analysis there must be no delay, because apomorphine is easily decomposed, especially when the solution is alkaline. When apomorphine is absent, examine the solution at once for morphine and narceïne.

Before apomorphine, morphine and narceïne can be extracted with the proper solvent, the alkaline, aqueous solution separated from ether must be rendered alkaline with ammonium hydroxide solution. First, acidify the solution with dilute hydrochloric acid (test with litmus paper) and then add ammonium hydroxide solution until alkaline. If the appearance of the solution has given any indication of apomorphine, as described above, then it should be first extracted with ether. On the other hand, if there be no reason to suspect apomorphine, the solution should be directly extracted several times with boiling chloroform. (See below.)

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[1] Ether will dissolve traces of morphine when the alkaloid is freshly precipitated and in an amorphous condition.

[2] Although antipyrine and caffeïne are freely soluble in chloroform, they dissolve with difficulty in ether. The latter solvent frequently fails to extract these substances completely from aqueous solution. They will then appear also in the chloroform extract.

When apomorphine is present, the residue from ether is an amorphous or crystalline powder having a greenish color.

APOMORPHINE.

Apomorphine, $C_{17}H_{17}NO_2$, is freely soluble in ethyl alcohol, amyl alcohol, ether and chloroform. The moist or dissolved alkaloid is especially susceptible to oxidizing influences and soon turns green. This green oxidation product is soluble in ether, benzene and chloroform with a red or violet color. Apomorphine, without preliminary treatment with hot, concentrated hydrochloric or sulphuric acid, gives directly those tests, described under morphine, which depend upon the conversion of morphine into apomorphine. This alkaloid like morphine reduces iodic acid solutions.

Apomorphine may be recognized by the following tests:

1. **Husemann's Test.**—Dissolve apomorphine in concentrated sulphuric acid and add a drop of concentrated nitric acid. An evanescent, violet color will appear and soon change to blood-red and later to yellowish-red.

2. **Pellagri's Test.**—Dissolve apomorphine in dilute hydrochloric or sulphuric acid and first neutralize the solution with acid sodium carbonate. Then add 1 to 4 drops of an alcoholic solution of iodine and shake thoroughly for several minutes. The solution will have a blue-green or emerald-green color. Extract with ether and the solvent will have a violet color.

3. **Test with Froehde's Reagent.**—This reagent dissolves pure apomorphine with a green color. When the

alkaloid has been acted upon by air to any extent, the color will be violet.

4. **A. Wangerin's[1] Test.**—Prepare a fresh solution of apomorphine hydrochloride (about 1 per cent.). Add 4 drops of potassium dichromate solution (0.3 per cent.) to 1 c.c. of this solution and shake for 0.5 to 1 minute. The solution will have an intense, dark-green color. Then add 10 c.c. of acetic ether and shake vigorously. This solvent will have a beautiful, violet color. Finally, add from a pipette about 5 drops of stannous chloride solution (1 per cent.) and again shake thoroughly. The color of the acetic ether layer will change to green. Prepare stannous chloride solution (1 per cent.) from 1 gram of crystallized stannous chloride ($SnCl_2.2H_2O$), 50 c.c. of hydrochloric acid (25 per cent.) and 50 c.c. of water. If 10 c.c. of chloroform be substituted for acetic ether in this test, the oxidation product of apomorphine will impart the same violet color to the chloroform. But if the stannous chloride solution be added carefully, the color will change to a pure indigo-blue.

MORPHINE.

Morphine, $C_{17}H_{19}NO_3$, can be detected by the following preliminary test. Add dilute sulphuric acid in slight excess to a portion of the alkaline solution, then a few c.c. of iodic acid solution and finally a small quantity of chloroform. Shake the mixture vigorously. A violet color in the chloroform caused by free iodine indicates morphine. This test, however, is not a positive indication of morphine, since many other substances besides this alkaloid will reduce iodic acid. In examining parts of cadavers, free from morphine and showing no sign of putrefaction, the author has nearly always obtained extracts which have very strongly reduced iodic acid. Consequently, this preliminary test with iodic acid is to be regarded merely as a delicate indication of morphine.

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[1] *Pharmaceutische Zeitung*, 47, 739/740 (1902).

To identify morphine positively, place the aqueous solution,[1] made alkaline with ammonium hydroxide solution as previously directed (see page 96), in a large flask and extract at once with a considerable quantity of hot chloroform. Withdraw the solvent by means of a separating funnel in the usual manner. The aqueous solution must be extracted 3 or 4 times with fresh portions of hot chloroform, because the free base is only slightly soluble in this solvent. Chloroform and alkaline solutions sometimes form emulsions which separate only with great difficulty. In this case gently warm the mixture upon the water-bath, and shake cautiously from time to time. This will soon cause the chloroform layer to separate clear. Pour the combined chloroform extracts into a dry flask, and add a few crystals of desiccated sodium chloride to remove adherent water. Finally, pass the clear chloroform solution through a dry filter. Set a small glass dish upon the water-bath, and add the chloroform solution drop by drop as fast as the solvent evaporates. Since the first chloroform extract usually does not give as pure morphine as the succeeding portions, it is advisable to collect the latter separately. Froehde's, Pellagri's, Husemann's and the formaldehyde-sulphuric acid tests should be made with these chloroform residues. The presence of morphine in the material is definitely established only when all these tests are positive! If the quantity of residue permit, morphine should also be identified by the ferric chloride test, as this is especially characteristic of morphine. It requires, however, more than traces of the alkaloid.

Purification of the Chloroform Residue.—When the chloroform residue is very impure, its purification is absolutely necessary, especially when the impurities have a reddish or brownish color. Dissolve the residue in hot amyl alcohol, and extract thoroughly with hot water containing a few drops of dilute sulphuric acid. The acid will remove morphine, whereas the amyl alcohol will retain most of the coloring-matter. Add ammonium hydroxide solution in excess to the acid liquid, and extract morphine with several portions of hot chloroform. The residue from this chloroform extract will consist of nearly pure morphine.

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[1] Morphine can be extracted by hot chloroform, amyl alcohol or acetic ether only from a solution containing free ammonia. Free fixed alkalies (KOH, NaOH) must not be present!

Properties of Free Morphine.—Morphine crystallizes from dilute ethyl alcohol in colorless, transparent, shining prisms. It is only slightly soluble in water (1 : 5000 at 15° and 1 : 500 at 100°). Aqueous morphine solutions are alkaline and very bitter. Neither crystallized morphine, nor the alkaloid which has become crystalline, is soluble in ether and benzene. These solvents will however, dissolve small quantities of the freshly precipitated and amorphous alkaloid. Amyl alcohol, chloroform and acetic ether more easily dissolve the amorphous than the crystallized alkaloid. Ammonium hydroxide and the alkaline carbonates precipitate morphine from solutions of its salts first in the amorphous form which later becomes crystalline. The caustic alkalies dissolve morphine, and ammonium chloride will precipitate the alkaloid from such solutions. Concentrated sulphuric acid dissolves pure morphine without color.

Morphine is a tertiary base and at the same time a monacid phenol. It is the presence of a phenolic hydroxyl-group which renders morphine soluble in alkalies. Carbon dioxide, however, will decompose these salts and precipitate the free base. Morphine, moreover, contains an alcoholic hydroxyl-group.

Sulphuric, hydrochloric, phosphoric and oxalic acids, the alkalies, zinc chloride, etc., remove a molecule of water from morphine and convert it into apomorphine.

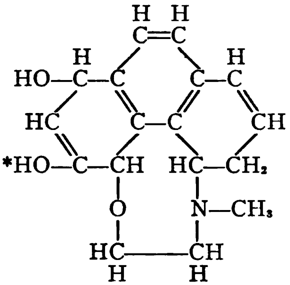
$$\underset{\text{Morphine.}}{C_{17}H_{19}NO_3} = \underset{\text{Apomorphine.}}{C_{17}H_{17}NO_2} + H_2O.$$

Morphine is very easily oxidized. Even atmospheric oxygen, as well as potassium permanganate, potassium ferricyanide, ammoniacal copper solution, etc., will oxidize the alkaloid in alkaline solution and convert it into a non-toxic substance which is soluble in alkalies. This oxidation product is oxy-di-morphine, also called pseudomorphine.

$$\underset{\text{Morphine.}}{2C_{17}H_{19}NO_3} + O = \underset{\text{Pseudomorphine.}}{(C_{17}H_{18}NO_3)_2O} + H_2O.$$

Morphine acts as a strong reducing agent, because of the ease with which it is oxidized. Morphine will reduce silver nitrate and gold chloride even in the cold.

The constitution of morphine has been determined with considerable certainty and is essentially the same as that of codeïne which is monomethyl-morphine. These two alkaloids are nitrogen derivatives of a tetrahydro-phenanthrene.



Vongerichten's Morphine Formula.

The hydrogen atom marked with an asterisk in the structural formula of morphine is replaced by a methyl-group ($—CH_3$) in the case of codeïne which is monomethyl-morphine.

Morphine may be recognized by the following tests:

1. Concentrated Nitric Acid Test.—Concentrated nitric acid dissolves morphine with a blood-red color which gradually changes to yellow. Stannous chloride solution does not produce a violet color in this solution. (Distinction from brucine.)

2. Husemann's Test.—Dissolve morphine in a watch-glass with a few c.c. of concentrated sulphuric acid, and heat the solution for 30 minutes upon the water-bath, or over a small flame for a very short time until white fumes arise. A reddish to brownish color will appear. Cool thoroughly and add 1 or 2 drops of concentrated nitric acid. A very fugitive, reddish-violet color will appear and soon change to blood-red or yellowish-red. This color gradually disappears.

It is better to dissolve morphine in cold, concentrated sulphuric acid and add a trace of nitric acid after the solution has stood 24 hours in a desiccator. A small crystal of potassium nitrate or potassium chlorate may be used instead of nitric acid.

In the examination of animal material, it is not unusual to obtain impure morphine from the chloroform extract. Such a residue will give a more or less highly colored solution with sulphuric acid. Heat will usually intensify the color. But even under these conditions, it is possible to detect the red color caused by nitric acid or potassium nitrate.

3. **Pellagri's Test.**—Perform this test as described for codeïne. (See page 89.) Avoid excess of alcoholic solution of iodine, otherwise the green color may be masked by the color of iodine.

4. **Test with Froehde's Reagent.**—This reagent dissolves morphine and its salts with a beautiful, violet color. This color gradually passes through blue to dirty green and finally changes to a faint red. Addition of water immediately destroys these colors. The blue color is usually very fugitive. This test may be made with a chloral hydrate solution of morphine.

5. **Formaldehyde-Sulphuric Acid Test.**[1]—The reagent should not be prepared until it is to be used. It is made by adding 2 or 3 drops of aqueous formaldehyde solution (containing about 40 per cent. of H.CHO) to 3 c.c. of pure, concentrated sulphuric acid. If a few drops of "formal-

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[1] *Zeitschrift für analytische Chemie*, 38, 466 (1899), and 40, 61 (1901).

dehyde-sulphuric acid" be mixed with a trace of morphine, it will produce a purple-red color which will change to violet, blue-violet and finally become nearly pure blue. If this blue solution be kept in a small test-tube and only slightly exposed to air, the color will persist for some time.

6. **Iodic Acid Test.**—Dissolve morphine in dilute sulphuric acid, and shake the solutions with a few drops of iodic acid solution and chloroform. If morphine be present, it will liberate iodine which will dissolve in chloroform with a violet color.

7. **Ferric Chloride Test.**—Add 1 or 2 drops of neutral ferric chloride solution[1] to a neutral solution of a morphine salt. A beautiful blue color will appear. In testing the chloroform residue, dissolve a considerable quantity in very dilute hydrochloric acid. Evaporate this solution to dryness upon the water-bath, dissolve the residue in pure water and add a drop of ferric chloride solution.

8. **Prussian Blue Test.**—Add a few drops of a dilute mixture of solutions of ferric chloride and potassium ferricyanide to a solution of a morphine salt. A deep blue color will appear. When considerable morphine is present, it will cause a precipitate of Prussian blue. This test is not characteristic of morphine.

9. **Silver Test.**—Warm a solution of a morphine salt with silver nitrate solution and an excess of ammonium hydroxide solution. Morphine will produce a gray precipitate of metallic silver.

Iodine-potassium iodide, phospho-tungstic acid, potassium mercuric iodide, potassium bismuthous iodide and phospho-molybdic acid are especially delicate, general reagents for morphine. Tannic acid solution will not produce an immediate precipitate, but gradually a faint turbidity will appear and increase upon standing. Chloride of platinum after some time will produce an orange-yellow, granular precipitate.

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[1] Add sodium carbonate solution to ordinary ferric chloride solution until it causes slight turbidity.

In animal metabolism morphine is subjected to changes which make its detection very difficult or impossible. Marquis,[1] as the result of experiments with cats, states that morphine after administration is present in the body in three forms, namely, as unaltered, as conjugate and as modified morphine.

The exact chemical nature of the substance which combines with morphine to form the conjugate alkaloid is still unknown. Evaporation with hydrochloric acid destroys the combination and the liberated morphine can then be identified.

Morphine strongly resists putrefactive changes. In parts of a cadaver (stomach and intestine with contents) containing morphine, the author[2] detected the alkaloid even after 18 months. During this time the organs, which had been kept in a glass jar where the supply of air was insufficient, had completely putrefied.

NARCEÏNE.

Narceïne, $C_{23}H_{27}NO_8.3H_2O$, crystallizes from water or alcohol in prisms which melt at 171° when they contain water of crystallization and at 145° when anhydrous. It has only a faintly bitter taste. Although only slightly soluble in cold water, it dissolves with ease in boiling water. The hot, saturated, aqueous solution solidifies and forms a thick, crystalline mass. This alkaloid is more readily soluble in aqueous solutions of the hydroxides of ammonium, sodium and potassium. Narceïne is insoluble in ether, benzene and petroleum ether. Moreover, it is soluble in cold ethyl alcohol, chloroform and amyl alcohol only with difficulty. Narceïne is a weak, tertiary base and optically inactive. Of all the opium alkaloids, narceïne has the most pronounced narcotic properties.

Narceïne may be recognized by the following tests:

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[1] *Arbeiten des Dorpater Institutes*, ed. Kobert, 14 (1896).

[2] *Berichte der Deutschen pharmaceutischen Gesellschaft*, 11, 494 (1901).

1. **Concentrated Sulphuric Acid Test.**—Concentrated sulphuric acid dissolves narceïne with a grayish-brown color. Upon standing for some time, also upon application of heat, this color changes to blood-red.

2. **Dilute Sulphuric Acid Test.**—When evaporated with dilute sulphuric acid, narceïne behaves like narcotine. (See page 90.)

3. **Test with Froehde's Reagent.**—At first a solution of narceïne in this reagent has a brownish-green color which gradually changes to green and finally to blood-red. Gentle heat hastens this color change.

4. **Iodine Test.**—Aqueous iodine solution (iodine water) or iodine vapor colors solid narceïne blue. Morphine interferes with or entirely prevents this reaction.

5. **Test with Erdmann's Reagent.**—This reagent, as well as concentrated sulphuric acid, dissolves narceïne with a yellow color which heat changes to dark orange.

6. **Chlorine-Ammonia Test.**—Pour a few drops of chlorine water upon narceïne and add, while stirring, a few drops of ammonium hydroxide solution. A deep red color will immediately appear.

7. **Resorcinol-Sulphuric Acid Test.**[1]—Mix thoroughly upon a watch-glass resorcinol (0.01 to 0.02 grams) with 10 drops of pure, concentrated sulphuric acid. Add a trace of narceïne (about 0.002 to 0.005 grams) and, while stirring, warm the intensely yellow solution upon a boiling water-bath. A carmine-red to cherry-red color will appear. As the solution cools, this color will begin at the edge to change gradually to more of a blood-red and finally after several hours to orange-yellow.

8. **Tannin-Sulphuric Acid Test.**—Mix narceïne (0.002 to 0.01 grams) with tannin (0.01 to 0.02 grams) and 10 drops of pure, concentrated sulphuric acid. Heat with

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[1] A. Wangerin, *Pharmaceutische Zeitung,* 47, 916 (1902).

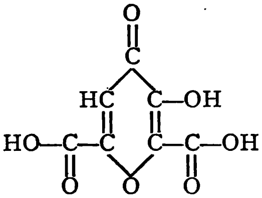
constant stirring upon the water-bath and the color of the solution, which is yellowish-brown at first, will soon become pure green. If heat be applied for some time, the green color will change to blue-green and finally to a dirty-green. "Tannin-sulphuric acid" gives a similar color test with narcotine and hydrastine which closely resemble narceïne in constitution. Narceïne differs from morphine and codeïne in not giving Pellagri's reaction.

APPENDIX OF GROUP II.

1. DETECTION OF OPIUM BY MECONIC ACID[1] AND MECONINE.

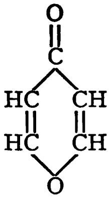
Since it is very easy to procure small quantities of opium preparations, especially the tincture, poisoning from this source becomes a possibility. Consequently, it is often important to recognize the presence of opium itself. Detection of the alkaloids, narcotine and morphine, which always occur in opium in considerable quantity, affords partial evidence of the presence of this substance. Moreover, opium always contains two non-basic substances, meconic acid and meconine. Detection of these two compounds in conjunction with narcotine and morphine definitely determines the presence of opium.

Meconic Acid.



Meconic Acid.

Meconic acid, or hydroxy-pyrone-dicarboxylic acid, crystallizes in plates or prisms with 3 molecules of water. It is easily soluble in hot water and alcohol. A solution of a ferric salt imparts to a solution of meconic acid a dark red color. Pyrone, of which meconic acid is a derivative, has the structure



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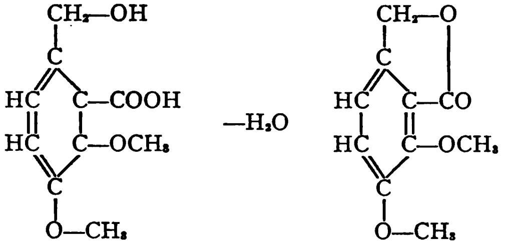
[1] Meconic acid can be distinctly recognized in urine after poisoning by opium and its preparations.

To detect meconic acid, extract a portion of the original material with alcohol containing a few drops of hydrochloric acid. Filter and evaporate the filtrate. Dissolve the residue in a small quantity of water, and heat the filtered solution to boiling with excess of calcined magnesium oxide. The solution will contain magnesium meconate. Filter to remove undissolved magnesium oxide, and acidify the filtrate faintly with dilute hydrochloric acid. Add a few drops of ferric chloride solution. A brownish-red to blood-red color will appear, when meconic acid is present. This red color will not disappear when the solution is warmed with hydrochloric acid, in which respect it differs from the red color caused by acetic acid.

Meconic acid can be distinctly recognized in an extract from 0.05 grams of opium.

Meconine.

Meconine, of which opium contains from 0.05 to 0.08 per cent., forms small prisms which melt at 102° and sublime without decomposition. It is easily soluble in ethyl alcohol, ether, benzene and chloroform, and less easily soluble in water. Alkalies convert meconine into easily soluble salts of meconinic acid, $C_{10}H_{12}O_5$. This monobasic acid cannot exist in the free condition but changes to meconine, when set free from its salts. Meconine, formed by abstracting a molecule of water from meconinic acid, is the lactone form of this acid.



Meconinic Acid. Meconine.

To detect meconine, extract the original material with alcohol containing sulphuric acid. Filter and evaporate the filtrate to a syrup. Dissolve the residue in considerable water, and extract meconine from this acid solution with benzene. Upon evaporating the solvent, meconine is frequently obtained in crystals. Meconine may be recognized by dissolving it in a small quantity of concentrated sulphuric acid. The solution will have a green color which will change to red within two days.

BEHAVIOR OF OPIUM ALKALOIDS WITH SELENIOUS ACID.

The reagent used in these tests is prepared by dissolving 0.5 grams of selenious acid (H_2SeO_3)[1] in 100 grams of pure, concentrated sulphuric acid. The reactions with morphine and codeïne are so distinctive that this reagent should be an excellent means of detecting opium. Its reactions are:

	Cold.	Hot.
Morphine:	Evanescent blue; then intense blue-green to persistent olive-green.	Brown.
Apomorphine:	Dark blue-violet.	Gradually dark brown.
Codeïne:	Blue quickly changing to emerald-green and later to persistent olive.	Steel-blue; then brown.
Narceïne:	Faint greenish-yellow; then violet.	Dark violet.
Narcotine:	Greenish steel-blue; later cherry-red.	Cherry-red.

2. DIGITALIS GLUCOSIDES.

Three distinct, crystalline glucosides with well-defined properties have been isolated from different parts of the digitalis plant (Digitalis purpurea). These compounds are digitalin (verum Kiliani), $C_{35}H_{56}O_{14}$, digitonin, $C_{37}H_{46}O_{14}$, and digitoxin, $C_{34}H_{54}O_{11}$. A fourth glucoside, digitaleïn, seems as yet not to have been obtained in an entirely pure condition.

Digitonin occurs almost exclusively in digitalis seeds. It crystallizes from alcohol in small needles and is soluble in 50 parts of 50 per cent. alcohol. Even very dilute hydrochloric acid decomposes this glucoside into digitogenin, dextrose and galactose.[2]

$$\underset{\text{Digitonin.}}{C_{37}H_{46}O_{14}} + H_2O = \underset{\text{Digitogenin.}}{C_{25}H_{24}O_3} + \underset{\text{Dextrose.}}{C_6H_{12}O_6} + \underset{\text{Galactose.}}{C_6H_{12}O_6}.$$

Digitonin is inactive and does not have a toxic effect upon the heart.

Digitoxin occurs almost exclusively in digitalis leaves. It is very active and nearly insoluble in water. It crystallizes from 85 per cent.

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[1] Mecke, *Zeitschrift für öffentliche Chemie,* 5, 351 and abstract in *Zeitschrift für analytische Chemie,* 39, 468 (1900) and *Chemisches Centralblatt,* 683 (1899, II).

[2] H. Kiliani, *Berichte der Deutschen chemischen Gesellschaft,* 24, 340 (1891).

alcohol in leaflets which melt at 145°. It is decomposed by alcoholic hydrochloric acid into digitoxigenin and digitoxose.

$$\underset{\text{Digitoxin.}}{C_{34}H_{54}O_{11}} + H_2O = \underset{\text{Digitoxigenin.}}{C_{22}H_{32}O_4} + \underset{\text{Digitoxose.}}{2C_6H_{12}O_4}.$$

H. Kiliani's[1] Test for Digitoxin.—Dissolve digitoxin in 3 or 4 c.c. of glacial acetic acid containing iron (100 c.c. of glacial acetic acid and 1 c.c. of 5 per cent. ferric sulphate solution) and cautiously add sulphuric acid containing iron (100 c.c. of sulphuric acid and 1 c.c. of 5 per cent. ferric sulphate solution) in about the same quantity, so that it forms an under layer. A dark zone will appear where the two solutions touch and the acetic acid above will gradually acquire a deep indigo-blue color.

Digitalinum verum, which occurs almost exclusively in the seed of Digitalis purpurea, is soluble in water (1:1000) and very active. In presence of hydrochloric acid, it is decomposed into digitaligenin, digitalose and dextrose.[2]

$$\underset{\substack{\text{Digitalinum}\\ \text{verum.}}}{C_{35}H_{56}O_{14}} + H_2O = \underset{\text{Digitaligenin.}}{C_{22}H_{30}O_3} + \underset{\text{Digitalose.}}{C_7H_{14}O_5} + \underset{\text{Dextrose.}}{C_6H_{12}O_6(?)}.$$

At present nothing definite is known regarding either the fate of the digitalis glucosides in animal metabolism, or the nature of the compounds into which they are changed, or the forms in which they are eliminated.

3. AQUEOUS CHLORAL HYDRATE SOLUTION AS A SOLVENT FOR ALKALOIDS, GLUCOSIDES, ETC., AND ITS USE IN TOXICOLOGICAL ANALYSIS.

RICHARD MAUCH.

(Communication from Professor E. Schaer's Institute, Strassburg.)

One part of water at 17.5° dissolves 4 parts of chloral hydrate, forming a very mobile solution which is easily filtered and capable of being kept for a long time without decomposition. This 80 per cent. solution of chloral hydrate easily dissolves relatively large quantities of alkaloids and glucosides without altering them chemically. At 17.5° one part of each of the following substances requires for solution the number of parts of solvent stated in the table:

---

[1] *Archiv der Pharmacie*, 234, 273–277 (1896).

[2] *Berichte der Deutschen chemischen Gesellschaft*, 31, 2454 (1898).

	Chloral Hydrate Solution. (80%)	Water.	Ether.	Chloroform.
Atropine:	5	600	50	3.5
Quinine:	6	2000	freely soluble	2
Cocaïne:	5	700	freely soluble	freely soluble
Morphine:	5	5000	1250	100
Santonin:	4	5000	125	4
Strychnine:	6.5	6600	1300	6
Brucine:	6.5	....	....	....
Veratrine:	7.5	....	....	....

Caffeïne is the only alkaloid which forms with chloral hydrate a molecular compound soluble in water. If a chloral hydrate solution of an alkaloid, which has been freshly prepared in the cold, be diluted with considerable water, the unchanged alkaloid will be precipitated almost quantitatively, for instance, morphine, strychnine and quinine. Substances like picrotoxin, santonin and acetanilide behave similarly. But when such solutions stand for a long time at ordinary temperatures, or are heated for 1 or 2 hours, chloral hydrate is decomposed by the vegetable base into chloroform and formic acid. Since the alkaloidal salts of formic acid are soluble in water, dilution with this solvent will not precipitate the alkaloids. R. Mauch has shown clearly that atropine, brucine, quinine, cocaïne, morphine, narcotine, strychnine and veratrine behave as just described.

In the tests ordinarily made with the ether or chloroform residue, R. Mauch recommends dissolving the residue in 80 or 60 per cent. chloral hydrate solution. The "chloral solution" should prove of great value in color tests which depend upon the use of pure sulphuric acid or sulphuric acid containing iron or molybdic acid. These solutions contain such a small quantity of water that it cannot modify the action of sulphuric acid upon the substance in solution. Such a "chloral solution" is also well adapted for zone-tests. An aqueous solution will form an upper layer with the "chloral solution," and the latter will form an upper layer with concentrated sulphuric acid.

Specific gravity of 80 per cent. chloral hydrate solution = 1.514.
" " " 60 " " " " = 1.3535.

In the tests ordinarily performed in test-tubes, it is best to use small tubes (6 or 7 cm. high; 1 cm. in diameter) holding 6 c.c. They should not be made of too thin glass. The chloral solution cannot be used in detecting picrotoxin, because chloral hydrate itself produces the same reduction changes caused by picrotoxin. The same is true of the test for strychnine, where sulphuric acid and potassium dichromate, or any other oxidizing agent, are used. Coniïne and nicotine also belong to the class of alkaloids which cannot be detected in chloral hydrate solution. Concentrated chloral hydrate solutions cannot be used directly in making tests with general, alkaloidal reagents, because precipitates will not appear until the solutions have been diluted with 6 to 8 volumes of very dilute hydrochloric or sulphuric acid.

In using the "chloral hydrate method" in toxicological analysis, the ether, chloroform or amyl alcohol extract should be evaporated with gentle heat upon a watch-glass of medium size (about 5 cm. diameter) and not too flat. Add to the residue, depending upon the quantity, about 3 c.c. of 75 per cent. chloral hydrate solution. Cover the glass and let it stand for some time. Occasionally tilt the glass and bring the solution thoroughly in contact with the residue. Pass the solution through a very small filter, if necessary, and wash both watch-glass and filter with a few drops of pure chloral hydrate solution. Use this chloral hydrate solution for the individual tests. In testing for strychnine, evaporate a part of the chloral solution to dryness upon the water-bath. Warm, until the residue does not smell of chloral, and then test for strychnine with sulphuric acid and potassium dichromate.

To recover from the chloral hydrate solution most of the alkaloids and substances like picrotoxin, acetanilide and phenacetine, add excess of sodium hydroxide solution and extract thoroughly with a small quantity of chloroform. The "chloral hydrate method" is conducive to very neat work and this is a great advantage. The use of metallic utensils like knives and spatulas is entirely unnecessary.

4. PTOMAÏNES.

Ptomaïnes are basic substances containing nitrogen which may be toxic or non-toxic. They are produced during putrefaction of cadavers under the influence of bacteria. They are to be regarded to some extent as products of bacterial metabolism and are nearly always present in cadavers, especially in those parts which are in an advanced state of putrefaction. Many ptomaïnes closely resemble alkaloids. Like alkaloids they give precipitates with the general reagents, and certain ptomaïnes resemble well-defined alkaloids even with special reagents. Hence ptomaïnes are of very great importance in forensic chemistry, since their presence may easily lead to mistakes and false conclusions. These putrefactive products also resemble vegetable bases in their behavior with solvents. Ether extracts some of them from acid solution and others from alkaline solution, whereas certain ptomaïnes are removed from alkaline solution only by amyl alcohol or chloroform. Most of the ptomaïnes are strong reducing agents, for example, they will immediately convert potassium ferricyanide into ferrocyanide. Consequently, they give the Prussian blue test with a dilute mixture of solutions of ferric chloride and potassium ferricyanide. Many of the alkaloids like morphine resemble the ptomaïnes in this respect.

The resemblance of a ptomaïne to a definite vegetable base is frequently confined to some one reaction, and never extends to all the reactions characteristic of the particular alkaloid. In a legal-chemical investigation no precaution, which guards against mistaking a ptomaïne for a vegetable base, should be omitted. It is an invariable rule to make every test characteristic of the suspected alkaloid, and not to be satisfied with possibly one positive test. A determination of the physiological action of the substance should supplement the chemical examination. A ptomaïne may resemble a vegetable base chemically, and yet the two substances may differ very decidedly in physiological action. Thus far, ptomaïnes have been found which show certain resemblances to coniïne, nicotine, strychnine, codeïne, veratrine, delphinine, atropine, hyoscyamine, morphine and narceïne. Selmi has described a putrefactive product which resembles morphine. Ether failed to extract it, either from acid or alkaline solution, whereas amyl alcohol removed it with ease from an alkaline or ammoniacal solution. It liberated iodine from iodic acid, but failed to give the tests which are characteristic of morphine alone, namely, Husemann's, Pellagri's and the ferric chloride tests!

The object in such cases must be to get a result about which there can be no doubt. Every possible means must be used to isolate the alkaloid in a perfectly pure state. When this can be accomplished, the nature of the poison can always be established beyond question.

SYNOPSIS OF GROUP II.

STAS-OTTO METHOD.

A. RESIDUE FROM ETHER EXTRACT OF ACID SOLUTION MAY CONTAIN:

Picrotoxin: Very bitter. Reduces Fehling's solution with heat. Langley's test. Melzer's test: red color with benzaldehyde and sulphuric acid.

Colchicin: Yellowish and amorphous. Dissolves in concentrated nitric acid with a violet to brownish-red color which becomes orange-red with excess of sodium hydroxide solution. White precipitate with tannic acid.

Picric Acid: Yellow. Aqueous solution dyes wool and silk yellow but not cotton. Red color with concentrated potassium cyanide solution. Red color with ammonium sulphide solution.

Acetanilide: Indophenol test. Heated with potassium hydroxide solution and chloroform, gives phenylisocyanide. Saponify by heating with alcoholic solution of potassium hydroxide; extract aniline with ether; test for aniline with calcium hypochlorite solution.

Caffeïne: Evaporated with strong chlorine water, gives a yellowish-red residue which immediately turns purplish-red with a small quantity of ammonium hydroxide solution.

Salicylic Acid: Residue consists of small needles. Red color when heated with Millon's reagent. Violet color with ferric chloride. Yellowish-white precipitate with bromine water.

Antipyrine: Mild, bitter taste. Easily soluble in water. Red color with ferric chloride. Two drops of fuming nitric acid give a green color which more acid and heat change to red. White precipitate with tannic acid.

Phenacetine: Tasteless. Indophenol but not phenylisocyanide test. Yellow color with concentrated nitric acid. Soluble with yellow color when heated with dilute nitric acid.

B. Residue from Ether Extract of Alkaline Solution May Contain:

Coniïne: Yellow globules having a sharp odor. A cold, saturated, aqueous solution becomes milky when warmed. Evaporated with hydrochloric acid, gives crystals of coniïne hydrochloride of characteristic appearance.

Nicotine: Liquid. Remains dissolved in residual water, upon evaporation of ether. Tobacco odor. Long, ruby-red needles (Roussin's crystals) with ether solution of iodine.

Aniline: Globules with reddish or brownish color. (See page 47, "Synopsis of Group I" for further details.)

Veratrine: Soluble in cold, concentrated sulphuric acid with yellow color which changes to deep red with heat. Very stable red color when heated with concentrated hydrochloric acid. Weppen's test with cane-sugar and sulphuric acid or Laves' test with furfurol and sulphuric acid.

Strychnine: Small needles. Very bitter. Dissolved in sulphuric acid, gives an evanescent blue or blue-violet color with potassium dichromate.

Brucine: Soluble in concentrated nitric acid with blood-red color which soon changes to yellowish-red. Addition of dilute stannous chloride solution produces a beautiful violet color. Dissolved in very dilute nitric acid and added to concentrated sulphuric acid as an upper layer, gives a red zone.

Atropine: Vitali's test. (Other substances give this test.) Enlargement of the pupil.

Cocaïne: Precipitated by potassium hydroxide solution from not too dilute solutions of its salts as globules, which soon solidify and become crystalline. Potassium permanganate precipitates violet-red cocaïne permanganate. Cleavage of benzoic acid with concentrated sulphuric acid and detection of former. Produces anaesthesia upon the tongue.

Physostigmine: Evaporated with ammonium hydroxide solution, gives a blue residue soluble in alcohol with blue color. Contraction of the pupil.

Codeïne: Dissolved in concentrated sulphuric acid, gives a blue or blue-violet color when heated with potassium arsenate or a trace of ferric chloride solution. Soluble in Froehde's reagent with a yellowish-green color which changes to blue with heat. Dissolved in concentrated sulphuric acid, gives a purple-red color when warmed with a drop of cane-sugar solution.

Narcotine: Neutral reaction. Not bitter. Soluble in Froehde's reagent with a greenish color which changes to cherry-red with heat. Soluble in Erdmann's reagent with a red color.

Quinine: Resinous varnish from ether. Very bitter. Dissolved in dilute sulphuric acid, shows a marked blue fluorescence. Thalleïoquin test. Herapathite test.

Caffeïne: Concentric clusters of shining needles from ether. Faintly bitter. Quite easily soluble in water. Solution neutral. Detected as stated under A.

Antipyrine: Easily soluble in water. Solution neutral. Faintly bitter. Detected as stated under A.

Pyramidone: Frequently as small needles from ether. Easily soluble in water. Solution neutral. Ferric chloride gives a blue-violet color which soon changes to red-violet. Fuming nitric acid produces a blue to blue-violet color.

C. RESIDUE FROM ETHER EXTRACT OF AMMONIACAL SOLUTION MAY CONTAIN:

Apomorphine: Usually amorphous from ether and frequently has a greenish color. Gives Husemann's and Pellagri's tests at once. Dissolved in dilute sulphuric acid, reduces iodic acid. Dissolved in Froehde's reagent, gives a green or violet color.

D. RESIDUE FROM CHLOROFORM EXTRACT OF AMMONIACAL SOLUTION MAY CONTAIN:

Morphine: Very bitter. Husemann's, Pellagri's and Froehde's tests especially characteristic. Formaldehyde-sulphuric acid test. Neutral solutions of morphine salts give a blue color with ferric chloride. Strong reducing action. Reduces iodic acid.

Narceïne: Iodine solution colors the free base blue. Soluble in resorcinol-sulphuric acid with a yellow color which changes to carmine-red or cherry-red with heat.

Soluble in tannin-sulphuric acid with yellowish-brown color which heat changes to pure green.

Antipyrine and caffeïne may also appear in this residue (D), since chloroform is a far better solvent than ether for these substances.

CHAPTER III.

METALLIC POISONS—GROUP III.

Put a portion of the original material, previously finely chopped and well-mixed, into a large flask, and add enough water to produce a fluid mass. Add an equal quantity of pure, concentrated hydrochloric acid.[1] Finally, add 1 or 2 grams of potassium chlorate, shake well and set the flask upon a boiling water-bath.

The residue remaining, after removal of volatile poisons by steam-distillation, may also be used in this part of the analysis, since it must still contain poisonous metals, provided any be present. Nascent chlorine begins to act upon organic matter, when the contents of the flask reach a certain temperature. Add 0.3 to 0.5 grams of potassium chlorate at 5 minute intervals, and frequently shake the flask. Continue in this manner, until most of the organic matter has been dissolved, and the solution has a pale yellow color. Then add a final portion of potassium chlorate, and heat a few minutes, until there is no odor of chlorine. Dilute with considerable water, adding also a few drops of dilute sulphuric acid, and filter after the mixture has stood for some time. When there is not too great an excess of free hydrochloric acid, the filtrate can be saturated directly with hydrogen sulphide. Otherwise, the solution must be evaporated nearly to dryness in a porcelain dish upon the water-bath, to remove most of the hydrochloric acid. Frequently, this operation gives the solution a dark brown color, which can be discharged by adding a few crystals of potassium chlorate. This evaporation is always advisable in testing for traces of lead, cadmium and copper, because hydrogen sulphide fails to precipitate these metals completely from solutions containing too much hydrochloric acid. The residue upon the filter may contain silver chloride, lead sulphate and barium sulphate. It should be further examined, as directed under "Metallic Poisons IV" (see page 140).

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[1] Usually 5 to 10 c.c., or at most 15 c.c., of concentrated hydrochloric acid will be sufficient for laboratory experiments. Avoid too large an excess of hydrochloric acid!

Nascent chlorine, generated by heating a mixture of hydrochloric acid and potassium chlorate, destroys those animal or vegetable substances, which interfere with the analytical processes. Some of the organic material is oxidized and rendered soluble; the remainder becomes insoluble, and forms a white, amorphous precipitate. An insoluble, white residue, which resists the action of chlorine, always remains, even after the most thorough treatment with hydrochloric acid and potassium chlorate. This is especially true of animal matter like the parts of a cadaver. This treatment converts a portion of the organic matter into volatile compounds like chloranil, which have a sharp odor and attack the mucous membranes. Finally, hydrochloric acid and potassium chlorate dissolve those metallic poisons which are present. Several metals like mercury, silver, lead, copper and zinc are precipitated from solution by certain animal, or vegetable compounds, especially by the proteïn substances. These precipitates are usually very stable compounds, in which the metal cannot be detected by the ordinary tests without further manipulation. Nascent chlorine sets the metal free from such metallic proteïn compounds, and converts it into a soluble chloride. As a rule, the metal can easily be detected when in this condition, since the solution contains ions of the metal in question. In many instances, the metal in these organic compounds behaves like copper in potassium cuprous cyanide ($K_2Cu_2(CN)_4$). This salt dissociates in aqueous solution into

$$K_2Cu_2(CN)_4 = 2\dot{K} + Cu_2(CN)_4''$$

and, consequently, fails to give the tests for metallic copper or, to speak more correctly, for the copper ion. But, when heated with hydrochloric acid and potassium chlorate, this compound is converted into cupric chloride. This salt will give all the copper tests, because the solution contains $\ddot{Cu}$ ions.

$$CuCl_2 = \ddot{Cu} + 2Cl'.$$

Potassium chlorate, however, acts vigorously only in a strong hydrochloric acid solution. Therefore, this acid should always be in excess. Should the mass become too thick at any time during the heating, it may be diluted with water, or dilute hydrochloric acid. Moreover, the contents of the flask should be vigorously shaken during treatment with potassium chlorate. This will prevent a large quantity of potassium chlorate from collecting upon the bottom of the flask. Such an occurrence may cause an explosion, due to formation of the exceedingly unstable dioxide of chlorine (ClO_2). Parts of a cadaver, heated with hydrochloric acid and potassium chlorate, are dissolved comparatively quickly. An experiment was made, in which 100 grams of stomach and duodenum, 20 grams of stomach contents, 75 grams of kidney and 200 grams of liver, in all 395 grams of parts of a cadaver, were treated in the manner described. In the course of an hour nearly the entire mass was dissolved. The insoluble portion was collected upon a filter and washed. It was an amorphous, gummy mass, which had a yellowish-white color, was greasy to the touch and weighed 52 grams, when dried upon a porous, earthen plate. When dried at 100°, it weighed only 32 grams. In toxicological analysis, the author uses hydrochloric acid, having a specific gravity of 1.05 to 1.06 (10.5 to 12 per cent. HCl), which is saturated with hydrogen sulphide, and kept in a loosely stoppered bottle. This insures precipitation of the final traces of arsenic, which may be present even in the purest commercial acid. This acid, before being used, is passed through a filter free from ash, to remove precipitated sulphur.

Sonnenschein and Jesserich use pure, free chloric acid instead of potassium chlorate. Place the material in a large flask, and dilute with water. Add a few c.c. of chloric acid, and warm slowly and very cautiously upon the water-bath. As soon as the mass swells and becomes porous, gradually add small portions of hydrochloric acid. Even a considerable quantity of parts of a cadaver will dissolve in 2 or 3 hours. Water lost by evaporation must be occasionally replaced, otherwise the reaction may take place with explosive violence. In other respects, the product of the reaction should be treated as described in the preceding process.

Detection of Metals in the Filtrate.

The filtrate must be examined for:

Arsenic
Antimony
Tin
Mercury
Lead
Copper
Bismuth
Cadmium
Chromium
Zinc.

The method of analysis, in which hydrochloric acid and potassium chlorate are used, when followed carefully, usually yields a solution having a faint, yellow color, when filtered and freed as completely as possible from excess of hydrochloric acid. Transfer the solution to a flask, and heat upon the water-bath. Then pass pure, arsenic-free[1] hydrogen sulphide into the solution for 0.5 to 1 hour. Continue this treatment, until the solution is cold.[1] When the solution has been saturated with hydrogen sulphide, stopper the flask loosely, and let the solution stand several hours, best over night. If the solution have a distinct odor of hydrogen sulphide on the following day, the next step in the analysis may be taken. Otherwise, the solution must be saturated with hydrogen sulphide again. Collect the hydrogen sulphide precipitate upon a filter free from ash, and wash with hydrogen sulphide water. Reserve the filtrate, and test it

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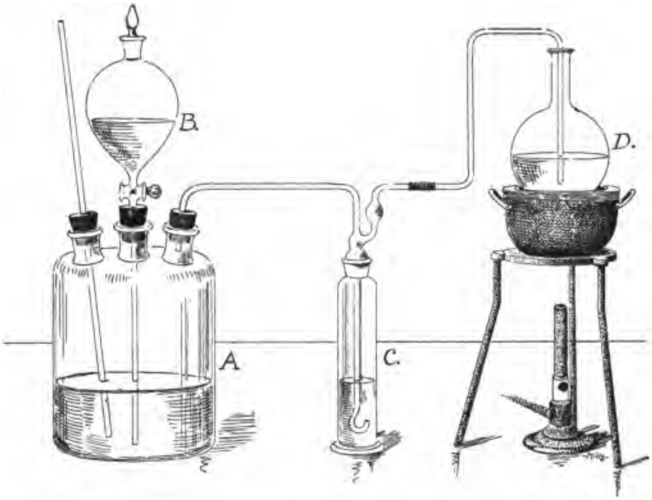
[1] Arsenic-free hydrogen sulphide is most easily prepared by saturating dilute sodium hydroxide solution with hydrogen sulphide, generated from crude iron sulphide and commercial hydrochloric acid. Pour this sodium hydro-sulphide solution into a separating funnel, and add

FIG. 10.



Apparatus for Generating Arsenic-free Hydrogen Sulphide.
(*a*) Generator with dilute sulphuric acid; (*b*) Separating funnel with NaSH; (*c*) Wash-bottle; (*d*) Solution to be saturated wlth H_2S.

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it slowly to dilute sulphuric acid. This will give arsenic-free hydrogen sulphide, the generation of which can be regulated according to need. (Fig. 10.)

[1] Chromium, in not too small quantity, will impart more or less of a green color, both to the solution and to the filtrate from the hydrogen sulphide precipitate.

for "Metallic Poisons III" (see page 137), namely, chromium and zinc. Both vegetable and animal material (parts of a cadaver), treated with hydrochloric acid and potassium chlorate, yield solutions which usually give precipitates with hydrogen sulphide. Such precipitates appear, even in the absence of metals of this group. They[1] have a yellow, or brown to brownish-red color, and consist to some extent of organic sulphur compounds. In an analysis of this description, it is impossible to tell from the color of the hydrogen sulphide precipitate, whether a metal is present or not.

Test for Complete Precipitation.—Before testing for chromium and zinc, make sure that hydrogen sulphide has caused complete precipitation. Add 10 to 20 times its volume of strong hydrogen sulphide water to a portion of filtrate from the hydrogen sulphide precipitate. If a colored precipitate fail to appear, metals of Group II have been entirely precipitated. Otherwise, dilute considerably the total filtrate from the hydrogen sulphide precipitate, and saturate it with hydrogen sulphide again. Lead, cadmium and also copper, to some extent, are not completely precipitated by hydrogen sulphide, especially in presence of too much dilute hydrochloric acid.

Extract the hydrogen sulphide precipitate, while still moist, with a hot mixture of approximately equal parts of ammonium hydroxide and ammonium sulphide solutions. This may be accomplished by passing the mixed reagents through the filter several times.

The filtrate, thus obtained, must be tested for "Metallic Poisons I" (see below), namely:

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[1] Thoroughly washed caseïn and fibrin are completely dissolved, after repeated treatment with hydrochloric acid and potassium chlorate. Hydrogen sulphide, passed into the filtrate from such a solution, will produce amorphous precipitates of a dirty yellow to brownish color. These precipitates contain organic sulphur compounds in addition to a large quantity of sulphur.

Arsenic
Antimony
Tin
Copper.[1]

The residue upon the filter must be tested for "Metallic Poisons II" (see page 135), namely:

Mercury
Lead
Bismuth
Copper
Cadmium.

METALLIC POISONS I.

Detection of Metals Whose Sulphides are Soluble in Ammonium Hydroxide and Sulphide.

Antimony
Arsenic
Tin
Copper.

Evaporate the ammonium sulphide filtrate, which usually has a dark brown color, caused by organic substances,[2] in a porcelain dish to dryness upon the water-bath. Moisten the residue with fuming nitric acid, and evaporate again. The residue, thus obtained, must be thoroughly mixed in a mortar with three times the quantity[1] of a mixture, composed of 2 parts of sodium nitrate (not potassium nitrate!) and 1 part of dry sodium carbonate. Thoroughly dry this mixture, and gradually add it in small portions to a small quantity of fused sodium nitrate in a porcelain crucible. Continue heating the crucible, after the final portion has been added, until the fused mass is a colorless liquid. If copper be present, copper oxide will impart a gray or grayish-black color to the melt. It may contain arsenate, pyro-antimonate and stannate of sodium, together with stannic and cupric oxides. Cool the melt, soften with hot water and transfer to a flask without loss. Add a small quantity of acid sodium carbonate to the resulting solution, whether clear or turbid. This will convert sodium hydroxide into sodium carbonate, and completely precipitate stannic oxide by decomposing the small quantity of sodium stannate which may be in solution. Finally, filter and make a separate examination of filtrate and residue.

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[1] Copper sulphide is quite soluble in hot, yellow ammonium sulphide. Copper oxide, formed by treating an ammonium sulphide solution containing copper, according to Meyer's method, imparts more or less of a gray appearance to the fused mass. Extraction of the fused mass with water leaves a residue, which may contain black oxide of copper, together with stannic oxide and sodium pyro-antimonate. To detect copper, dissolve the black residue in a small quantity of hot, dilute hydrochloric acid, and divide the solution into two parts. Add ammonium hydroxide solution to one portion, until alkaline. The solution should have a blue color. Add potassium ferrocyanide solution to the other portion. A brownish-red precipitate of copper ferrocyanide ($Cu_2Fe(CN)_6$) should appear.

[2] In toxicological analysis, hydrogen sulphide, passed into the hydrochloric acid solution, usually produces a dark precipitate even in the absence of metals. This precipitate, which is caused by organic substances, dissolves in most cases in hot ammonium sulphide with a dark brown color.

A. The filtrate will contain any arsenic in the form of sodium arsenate.

B. The residue may consist of sodium pyro-antimonate, stannic and cupric oxides.

There is usually an insoluble residue, even when these substances are absent. This may be caused by the crucible, the glazing of which is somewhat affected by fusion.

A. DETECTION OF ARSENIC IN THE FILTRATE.

Arsenic is usually detected by isolating the metal in its elementary condition. This affords the most positive proof of its presence. Of the various methods leading to this result, two in particular have found a place in forensic chemistry. These are, namely:

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[1] In the analysis of materials for practice, 3 grams of a mixture, containing 2 grams of sodium nitrate and 1 gram of sodium carbonate, will be sufficient in most cases. Avoid too large an excess of sodium nitrate!

1. **The Berzelius-Marsh Method.**
2. **The Fresenius-Von Babo Method.**

Each method is exceedingly accurate and precludes confusion of arsenic with antimony.

A full description of the exceedingly delicate and characteristic biological test for arsenic by means of moulds, especially Penicillium brevicaule, is given upon page 142.

1. BERZELIUS-MARSH METHOD.

The principle of this method is the conversion by nascent hydrogen of oxygen compounds of arsenic into hydrogen arsenide, AsH_3, (arsine), from which metallic arsenic can be isolated with comparative ease.

Procedure.—Filtrate A (see page 124), which contains any arsenic in the form of sodium arsenate, is treated with excess of arsenic-free sulphuric acid, and evaporated in a porcelain dish. To remove completely nitric and nitrous acids from the residue, add a few drops more of pure sulphuric acid, and heat the contents of the dish upon an asbestos plate, until copious, white fumes of sulphuric acid are evolved. The residue[1] in the porcelain dish is usually a thick, colorless liquid with a strong acid reaction. It contains arsenic in the form of arsenic acid and, when cold, it frequently solidifies to a white, crystalline mass. When diluted with water and thoroughly cooled, the solution is in proper condition to be examined in the Marsh apparatus for arsenic.

Place 30 to 40 grams of pure, arsenic-free zinc (granulated or in small rods) in flask A of the Marsh apparatus

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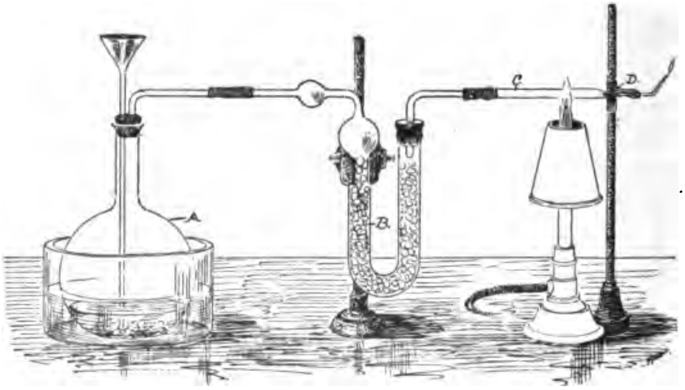
[1] To insure complete removal of nitric acid, test a few drops of this residue with ferrous sulphate and sulphuric acid, or with indigo solution and sulphuric acid.

(Fig. 11). Pour some cold, dilute, arsenic-free sulphuric acid upon the metal. Prepare this acid by mixing 1 volume of pure, concentrated sulphuric acid with 5 volumes of water. Such an acid will contain 15 or 16 per cent. of H_2SO_4. The temperature of the solution, which should not rise much during the analysis, can be controlled by generating hydrogen slowly. Otherwise, there is danger of partial reduction of sulphuric acid to hydrogen sulphide, which will interfere more or less with the detection of arsenic. Should the acid in the generator become too warm, cool the flask by immersion in a dish of cold water. In using the Marsh apparatus, certain precautions should be taken.

FIG. 11.



Marsh Apparatus.
(*a*) Hydrogen-generator; (*b*) Chloride of calcium drying-tube; (*c*) Hard glass tube; (*d*) Arsenic mirror.

1. Have the apparatus absolutely tight.

2. Do not ignite the hydrogen, until air has been entirely expelled. To tell when this point is reached, collect the hydrogen in a dry test-tube, until it ignites without detonation when carried to a flame. When hydrogen stands this test, it may be ignited at the tip of the ignition-tube without danger of explosion within the apparatus!

3. Test the hydrogen, to insure its entire freedom from arsenic. It should give neither the arsenic mirror nor spot.

If the hydrogen be free from arsenic, introduce the perfectly cold solution, containing arsenic as arsenic acid, into the generator in small portions. If the solution contain arsenic, the gas generated will consist of a mixture of arsine and hydrogen, in which case a shining mirror of metallic arsenic will appear, often in a few minutes, just beyond the point of ignition. When mere traces of arsenic are present, considerable time must elapse before a brown or brownish-black film will appear. Arsine, passed through a tube heated to redness, is decomposed into arsenic and hydrogen.

$$4AsH_3 = As_4 + 6H_2.$$

If heat be removed from ignition-tube C, the hydrogen flame will have a bluish-white color when arsenic is present. At the same time, white fumes of arsenious oxide (As_2O_3) will arise from the flame. Arsine has been completely oxidized.

$$2AsH_3 + 3O_2 = As_2O_3 + 3H_2O.$$

If a cold porcelain dish be depressed upon this flame, a lustrous, brownish-black spot (arsenic spot) will be deposited. In this case, arsine is burning in an insufficient supply of air, the result being precipitation of arsenic upon the cold porcelain.

$$4AsH_3 + 3O_2 = \underset{\text{Arsenic Spot.}}{As_4} + 6H_2O.$$

Arsine can be easily recognized also, by its exceedingly characteristic, garlic-like odor, which can be detected by extinguishing the hydrogen flame and allowing the gas to escape.[1] Even traces of arsine, mixed with hydrogen, are soon recognized by this odor.

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[1] The poisonous properties of arsine would seem to make such confirmation of its presence undesirable, the more so as arsine can be detected better by other tests. Tr.

Arsenic can be detected in a third way by the Marsh apparatus. Extinguish the hydrogen flame, and pass the gas into dilute silver nitrate solution. Arsine will darken the silver solution, and a black precipitate of metallic silver will soon appear. The solution will contain arsenious acid and free nitric acid.

$$AsH_3 + 6AgNO_3 + 3H_2O = 3Ag_2 + 6HNO_3 + H_3AsO_3.$$

Filter to remove silver and carefully neutralize the filtrate with ammonium hydroxide solution. It is possible to obtain a yellowish-white precipitate of silver arsenite (Ag_3AsO_3), which is readily soluble in ammonium hydroxide solution and in nitric acid.

Differences Between Arsenic and Antimony Spots and Mirrors.—Nascent hydrogen reduces various antimony compounds (for example, $SbCl_3$, Sb_2O_3, $HSbO_3$, tartar emetic, etc.), and produces stibine which behaves very much like arsine in the Marsh apparatus, that is to say, it gives a spot and mirror, and precipitates black silver antimonide (Ag_3Sb), when passed into silver nitrate solution.

Meyer's method of procedure sharply separates arsenic from antimony, and excludes the possibility of the two metals appearing at the same time in the Marsh test. The differences, however, between arsenic and antimony should be pointed out, since arsenic mirrors and spots must always be further identified by other tests. When antimony is suspected, it is frequently necessary to make further confirmatory tests (see under Antimony, page 134) by introducing the solution into the Marsh apparatus, and producing the antimony spot and mirror.

The essential differences between the arsenic and antimony spots and mirrors are:

(*a*) The arsenic mirror is lustrous and decidedly metallic. It is brownish-black and volatile. Owing to this latter property, it can be easily sublimed, when heated in a stream of hydrogen. In the antimony mirror the metal fuses and is silver-white, where it is in contact with the heated glass, but in those places, removed from the flame, it is almost black and has hardly any lustre. Stibine will decompose at a temperature much lower than that required for arsine. Consequently, some antimony is always deposited before the heated part of the tube is reached. Antimony volatilizes at a high temperature, and is, therefore, less easily sublimed.

(*b*) When the arsenic spot is not too heavy, it has a brownish-black, or a beautiful brown color, and is lustrous. It is readily soluble in sodium hypochlorite solution.

The antimony spot is not lustrous, but has a dull, velvet-black color. A thin film of antimony is never brown, but has a dark and graphite-like appearance. It is insoluble in sodium hypochlorite solution.

(*c*) A drop of concentrated nitric acid, or moist chlorine, easily dissolves the arsenic spot forming arsenic acid. Addition of silver nitrate solution, and neutralization with ammonium hydroxide solution, produce a reddish precipitate of silver arsenate (Ag_3AsO_4).

Nitric acid, or moist chlorine, will dissolve the antimony spot also. But silver nitrate will not produce a colored precipitate.

(*d*) An arsenic mirror, gently heated in the ignition-tube in a stream of dry hydrogen sulphide, can be changed to yellow arsenic trisulphide. Under the same conditions, an antimony mirror is colored brownish-red (Kermes color) to black, and antimony trisulphide is formed.

(*e*) Arsine and stibine differ also in behavior toward silver nitrate solution. In both cases, black precipitates are formed. Arsine, however, precipitates only metallic silver, and arsenious acid can be detected in the filtrate from this precipitate. On the other hand, stibine precipitates silver antimonide (Ag_3Sb), and the filtrate does not contain a trace of antimony, since the precipitation of black Ag_3Sb is complete. To detect antimony, boil the black precipitate for some time with 10 to 15 per cent. tartaric acid solution. Antimony will be completely dissolved, whereas silver will remain as a grayish-white residue. If dilute hydrochloric acid be added to this solution, hydrogen sulphide will precipitate antimony as orange-red antimony trisulphide.

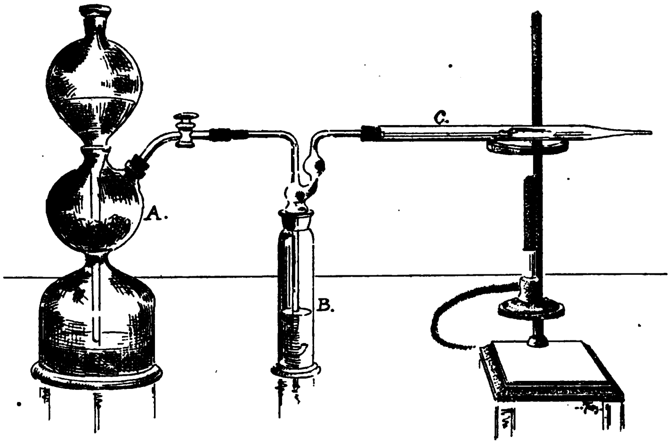
2. FRESENIUS-VON BABO METHOD.

The principle of this method is the reduction of oxygen and sulphur compounds of arsenic by fusion with a mixture of sodium carbonate and potassium cyanide, which produces a mirror of arsenic.

$$2As_2O_3 + 6KCN = As_4 + 6KOCN.$$
$$2As_2S_3 + 6KCN = As_4 + 6KSCN.$$

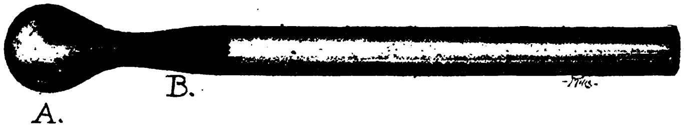
The solution prepared by Meyer's method contains arsenic in the form of arsenic acid. To reduce the arsenic acid to arsenious acid, add a few c.c. of sulphurous acid to the solution and heat, until there is no odor of this acid. Dilute this solution with water, and pass hydrogen sulphide through it. Collect the precipitate of arsenic trisulphide (As_2S_3) upon a small filter, and wash thoroughly. Dissolve the precipitate upon the filter in a small quantity of hot ammonium hydroxide solution. Evaporate this solution in a porcelain dish, and heat the residue with concentrated nitric acid. When the latter has been evaporated completely, moisten the residue with a few drops of water, and add enough dry sodium carbonate to make the mass distinctly alkaline. Thoroughly dry this residue, and mix in a mortar with several times the quantity of a mixture of 3 parts of sodium carbonate and 1 part of potassium cyanide. Transfer this mixture to a porcelain boat, and place in an ignition-tube of hard glass. Heat in a stream of carbon dioxide, and arsenic in the mixture will be reduced to the metallic condition. (Fig. 12.) Dry the carbon dioxide by passing it through pure, concentrated sulphuric acid. To remove moisture, heat the ignition-tube gently at first, where the boat is, and afterwards ignite at a bright red heat. A mirror of arsenic will appear upon the cooler part of the tube. It should be examined further, as already described.

FIG. 12.



Fresenius-Von Babo Apparatus.
(*a*) Carbon dioxide generator; (*b*) Drying-bottle with pure, concentrated sulphuric acid; (*c*) Ignition-tube and boat.

FIG. 13.



(*A*) Substance and fusion-mixture; (*B*) Arsenic mirror.

A simpler method of detecting arsenic, by means of potassium cyanide, consists in heating the perfectly dry material, containing arsenic (As_2O_3, As_2S_3), in a bulb-tube with a dry mixture of sodium carbonate and potassium cyanide, until fusion takes place. When the tube has been made smaller above the bulb, the arsenic mirror will form in the constricted area (Fig. 13).

Other Arsenic Tests.

1. **Bettendorff's Test.**—(Arsenic Test of German Pharmacopoeia, IV edition.) This test is based upon the fact that a concentrated stannous chloride solution will precipitate metallic arsenic from arsenious acid in the cold, and from arsenic acid with heat. In this test, it is advisable to use stannous chloride solution, prepared according to the directions of the Pharmacopoeia.[1] When only traces of arsenic are present, the solution has a red to brownish-red color. More than traces of arsenic produce a black precipitate of arsenic.

$$2As_2O_3 + 6SnCl_2 + 12HCl = As_4 + 6SnCl_4 + 6H_2O.$$

The solution mentioned elsewhere, which contains arsenic in the form of arsenic acid, may be used for this test. It is not as delicate as the Marsh test.

2. **Gutzeit's Test.**—(Arsenic Test of German Pharmacopoeia, II edition.) Very slight traces of arsenious and arsenic acids, as well as their salts, can be recognized with certainty by Gutzeit's[2] test. Hydrogen is generated in a test-tube from arsenic-free zinc and pure, dilute hydrochloric acid. To remove sulphurous acid which may be present, or hydrogen sulphide, add a few drops of iodine solution, until the liquid has a yellow color. Then add the solution to be tested, and place a loose, cotton plug in the neck of the test-tube. Put a paper cap, moistened with concentrated silver nitrate solution (1 : 1), over the mouth of the test-tube. If arsenic be present, the silver nitrate spot will become lemon-yellow. A compound of silver arsenide and silver nitrate is formed.

$$AsH_3 + 6AgNO_3 = \underset{\substack{\text{lemon-yellow} \\ \text{compound.}}}{(3AgNO_3 + Ag_3As)} + 3HNO_3.$$

A brownish-black border gradually forms around the yellow spot. A drop of water immediately turns the spot black, owing to separation of metallic silver.

$$(3AgNO_3 + Ag_3As) + 3H_2O = 6Ag + H_3AsO_3 + 3HNO_3.$$

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[1] For the preparation of this solution, see Appendix, page 217, and also U. S. Pharmacopoeia, VII revision, page 480.

[2] Gutzeit, *Pharmaceutische Zeitung,* 1879, 263, and Poleck and Thümmel, *Berichte der Deutschen chemischen Gesellschaft,* 16, 2438 (1883).

This is a very delicate test for arsenic. One drop of 0.1 per cent. potassium arsenite solution produces a distinct yellow color upon the silver paper. Even 0.00005 grams of As_2O_3 can be detected by Gutzeit's test.

Gutzeit's test, however, is not as characteristic of arsenic as the Marsh test. Stibine, phosphine from phosphorus in the zinc, and even hydrocarbons will color the silver nitrate paper. Dry hydrogen sulphide also produces a yellow or yellowish-green spot upon paper, moistened with concentrated silver nitrate solution. This spot has a black border which gradually extends, until the entire spot becomes black. Poleck states that this yellow compound has the composition ($AgNO_3 + Ag_2S$).

B. DETECTION OF ANTIMONY, TIN AND COPPER IN THE RESIDUE.

Residue B (see page 124), insoluble in water and obtained from the fusion, may contain sodium pyro-antimonate, stannic and cupric oxides. Treat this residue upon the filter with a small quantity of hot, rather dilute hydrochloric acid (equal parts of concentrated acid and water). Repeatedly pass this acid through the paper, until most of the residue is dissolved. If the original color of residue B was gray or black, first examine a portion of the hydrochloric acid solution for copper. Excess of ammonium hydroxide solution will produce a blue color, or potassium ferrocyanide solution will give a brownish-red precipitate, or only a coloration when traces of copper are present.

Concentrate the remainder of the hydrochloric acid solution to a few drops in a porcelain dish upon the water-bath, and put 2 drops of this solution upon platinum foil in contact with zinc. Antimony will produce a black spot, tin a grayish spot and copper a dark reddish-brown spot upon platinum. Neither the tin nor the copper spot can easily be confused with that given by antimony.

Dilute the remainder of the hydrochloric acid solution with water, and introduce a piece of zinc into the solution. Keep the zinc in the solution, as long as hydrogen is evolved. Collect the black, metallic flocks, which result from this operation, upon a small filter. Wash thoroughly, and gently warm these flocks with a small quantity of concentrated hydrochloric acid. Finally, filter the solution. Antimony does not dissolve, whereas tin passes into solution as stannous chloride ($SnCl_2$), and is in the filtrate.

Tin may be recognized by the following tests:

(*a*) **Mercury Test.**—Add a few drops of mercuric chloride solution to a portion of the filtrate. Tin will precipitate white mercurous chloride (calomel). Application of heat will produce also gray, metallic mercury, in case stannous chloride is present in large excess.

(*b*) **Prussian Blue Test.**—Add a few drops of a dilute mixture of ferric chloride and potassium ferricyanide solutions to another portion of the filtrate. Tin will produce a precipitate of Prussian blue.

Antimony may be further identified by dissolving the black flocks, which are insoluble in hydrochloric acid, in a few drops of aqua regia. Evaporate excess of acid upon the water-bath, and dilute the residue with water. When the quantity of antimony is not too small, water will precipitate antimony oxychloride ($O{=}Sb{-}Cl$). Redissolve this precipitate in a small quantity of dilute hydrochloric acid. Test a portion of this solution for antimony with hydrogen sulphide. Introduce the remainder into the Marsh apparatus, and produce the antimony spot and mirror, or test for stibine with silver nitrate solution, as has been described. (See page 129.)

METALLIC POISONS II.

DETECTION OF METALS WHOSE SULPHIDES ARE INSOLUBLE IN AMMONIUM SULPHIDE.

Mercury **Bismuth**
Lead **Copper**
Cadmium.

That portion of the hydrogen sulphide precipitate, insoluble in ammonium sulphide solution, may contain mercury, lead, bismuth, copper and cadmium sulphides. Examine this precipitate according to the methods employed in qualitative analysis. A small precipitate may be treated repeatedly upon the filter with a few c.c. of warm, rather dilute nitric acid (1 volume of concentrated acid and 2 volumes of water). Mercuric sulphide will not dissolve, but the other sulphides will pass into solution as nitrates.

DETECTION OF MERCURY IN THE RESIDUE INSOLUBLE IN NITRIC ACID.

Always examine that portion of the hydrogen sulphide precipitate, insoluble in nitric acid, for mercury, even when it is not black! Treat this residue upon the filter with a small quantity of hot, somewhat diluted hydrochloric acid, in which a few crystals of potassium chlorate have been dissolved, and pass the acid through the paper several times. Evaporate the filtrate to dryness in a porcelain dish upon the water-bath, and dissolve the residue in 2 or 3 c.c. of water containing hydrochloric acid. Filter this solution, and examine the filtrate for mercury.

(*a*) **Stannous Chloride Test.**—Add a few drops of stannous chloride solution to a portion of the filtrate. A white precipitate of mercurous chloride (calomel) will appear, when mercury is present. Excess of stannous chloride, especially if heat be applied, will reduce this precipitate to gray, metallic mercury.

(*b*) **Copper Test.**—Put a few drops of the filtrate upon a small piece of bright copper. Mercury will immediately deposit a gray spot which will have a silvery lustre when rubbed. Wash the copper, upon which mercury has been deposited, successively in water, alcohol and ether. Dry thoroughly and heat in a small bulb tube of hard glass. Mercury will sublime and collect in small, metallic globules on the cool sides of the tube. A trace of iodine vapor, introduced into the tube, will immediately transform the gray sublimate into scarlet mercuric iodide.

(*c*) **Phosphorous Acid Test.**—Add some phosphorous acid to another portion of the filtrate and warm gently. A white precipitate of mercurous chloride (calomel) will appear, when mercury is present.

(*d*) **Precipitation of Mercuric Iodide.**—Add 1 or 2 drops of very dilute potassium iodide solution to the remainder of the filtrate. A red precipitate (HgI_2), readily soluble in excess of potassium iodide, shows mercury.

EXAMINATION OF THE NITRIC ACID SOLUTION.

The nitric acid solution may contain lead, bismuth, copper and cadmium nitrates. Evaporate this solution nearly to dryness in a porcelain dish, and dissolve the residue in a small quantity of hot water. If the solution contain lead, dilute sulphuric acid will produce a heavy, white precipitate of lead sulphate. Test the filtrate from this precipitate for bismuth, copper and cadmium.

(*a*) **Copper and Bismuth Tests.**—Add excess of ammonium hydroxide solution to the greater part of this filtrate. A blue color shows copper. Should a white precipitate appear also, it may be bismuthous hydroxide ($Bi(OH)_3$). To detect bismuth with certainty, dissolve this precipitate upon the filter in a few drops of dilute hydrochloric acid. Pour this solution into a large quantity of water. A white precipitate of bismuthous oxychloride (O=Bi—Cl) shows bismuth. As an alternative test, add stannous chloride solution to the hydrochloric acid solution, and then excess of sodium hydroxide solution. When bismuth is present, a black precipitate of metallic bismuth will appear.

(*b*) **Potassium Ferrocyanide Test.**—Potassium ferrocyanide solution will precipitate brownish-red copper ferrocyanide when copper is present. Traces of copper will give the solution a brownish-red color. After long standing, there will be a deposit of copper ferrocyanide.

(*c*) **Precipitation of Metallic Copper.**—A bright knife-blade, immersed for a short time in a copper solution, will be coated red by metallic copper.

To detect cadmium in presence of copper, add solid potassium cyanide to the blue solution, produced by ammonium hydroxide until the blue color is discharged. Then pass hydrogen sulphide through the solution. Cadmium will be precipitated as the yellow sulphide (CdS), whereas copper will remain in solution as $K_2Cu_2(CN)_4$, potassium cuprocyanide.

When copper is absent, test for cadmium by passing hydrogen sulphide at once into the ammoniacal solution.

METALLIC POISONS III.

DETECTION OF CHROMIUM AND ZINC.

The filtrate from the hydrogen sulphide precipitate must be tested for chromium and zinc. Concentrate the filtrate to one-half or one third its original volume, and divide this solution into two parts.

DETECTION OF ZINC.

Add enough ammonium hydroxide solution to render one-half the concentrated filtrate alkaline. This treatment usually gives the solution a dark color. Then add ammonium sulphide solution. This reagent almost always produces a precipitate, since solutions, prepared from animal and vegetable materials, usually contain iron compounds and phosphates of the metals of the earths and of the alkaline earths. When this precipitate has settled, add acetic acid until the solution has a faint acid reaction. Stir the mixture thoroughly, and allow it to stand for some time. The color of the precipitate will become lighter, because acetic acid dissolves sulphide of iron. Moreover, the phosphates are partly dissolved, except ferric phosphate ($FePO_4$), which is insoluble in acetic acid. Collect the precipitate upon a filter. Wash, dry and ignite precipitate and filter in a porcelain crucible. Before ignition, moisten the filter with concentrated ammonium nitrate solution. Extract the residue from ignition with 3 c.c. of boiling, dilute sulphuric acid. Filter, and divide the filtrate into two parts.

(*a*) Add sodium hydroxide solution in excess to one portion of the filtrate. Filter, to remove the white precipitate of ferric phosphate which usually appears, and add a few drops of ammonium, or hydrogen sulphide solution, to the clear filtrate. Zinc if present will give, especially upon warming, a white, flocculent precipitate of zinc sulphide.

(*b*) Add ammonium hydroxide solution in considerable excess to the second part of the filtrate. Filter, to remove ferric phosphate, and acidify the filtrate with acetic acid. Warm the solution, and pass hydrogen sulphide through it. Zinc if present will appear as the white sulphide.

(*c*) A further test for zinc may be made by dissolving the precipitate produced by ammonium or hydrogen sulphide (as described above in *a* and *b*), after it has been collected upon a filter and thoroughly washed, in a few drops of dilute hydrochloric acid. Boil until hydrogen sulphide has been expelled, and filter to remove precipitated sulphur. Add potassium ferrocyanide solution to the clear, cold filtrate. This will precipitate $Zn_2Fe(CN)_6$, zinc ferrocyanide, which is white, slimy and nearly insoluble in dilute hydrochloric acid.

DETECTION OF CHROMIUM.

To test for chromium[1] in the second part of the filtrate from the hydrogen sulphide precipitate, concentrate the solution in a porcelain dish to a small volume. Then add twice the quantity of potassium nitrate and sodium carbonate, until the reaction is decidedly alkaline. Finally, heat this mixture until perfectly dry. Add this dry residue in small portions to a small quantity of potassium nitrate fused in a crucible. In fusing a large quantity of material, it is advisable to use a large, bright, nickel crucible which is especially adapted for this operation. When fusion is complete, cool thoroughly, boil crucible and contents with water in a porcelain dish and filter the solution. Chromium will give the filtrate more or less of a yellow color. Even mere traces[2] of chromium will give the filtrate a yellow color. When the solution of the melt is colorless, it is unnecessary to test for chromium. To detect chromium with greater certainty when the filtrate is yellow, divide the solution into two portions and make the following tests:

(*a*) **Chrome Yellow Test.**—Add acetic acid in excess to one portion of the filtrate, and boil for some time to expel carbon dioxide and nitrous acid as completely as possible. Then add a few drops of lead acetate solution. A yellow precipitate of lead chromate ($PbCrO_4$, " Chrome Yellow ") will appear, when chromium is present. When the precipitate is mixed with considerable lead sulphate or chloride, the color is only yellowish. A white precipitate is due to $PBSO_4$, $PbCl_2$ or $Pb_3(PO_4)_2$. When the aqueous solution of the melt is colorless, such a precipitate is usually obtained.

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[1] In testing for metallic poisons, chromic oxide (Cr_2O_3) which is insoluble in acids may be disregarded, since it is not poisonous.

[2] Two drops of 10 per cent. potassium chromate solution (= 0.01 grams of K_2CrO_4) in 500 c.c. of water will produce a marked yellow color. Fifty c.c. of this solution will contain 0.001 grams of K_2CrO_4, which can still be recognized with certainty by the yellow color.

Potassium nitrate, which is always present in the melt, lead acetate and acetic acid, when brought together in solution, produce a distinct yellow color, in which a white precipitate may appear yellow. To eliminate this source of error, allow the precipitate to settle, collect upon a filter and wash thoroughly. If it be pure white, chromium is absent.

(*b*) **Reduction Test.**—Add sulphurous acid to the second portion of the filtrate. The yellow color will change to green, or greenish-blue, with formation of chrome alum. This is not as delicate as the preceding test.

METALLIC POISONS IV.

DETECTION OF BARIUM, LEAD AND SILVER IN THE RESIDUE FROM HYDROCHLORIC ACID AND POTASSIUM CHLORATE.

Thoroughly dry the residue, add three times the quantity of a mixture of 2 parts of potassium nitrate and 1 part of sodium carbonate and triturate in a mortar with the filter. Gradually introduce this mixture into a hot porcelain crucible. In this operation organic substances will be oxidized by potassium nitrate with considerable deflagration. Finally when all the material is in the crucible, add 0.25 to 0.5 grams more of potassium nitrate. Cool the melt, soften with hot water and pass carbon dioxide into the turbid liquid for several minutes. This treatment will convert caustic alkali into carbonate, and completely precipitate lead which may be in solution. Then heat the solution to boiling and let it settle for some time. Collect upon a filter the sediment, which may contain barium carbonate, lead carbonate and metallic silver. Silver will give the sediment a gray color. Even in the absence of these metals, there will usually be a residue upon the filter, which results in a measure from the corrosion of the crucible during fusion. Thoroughly wash the precipitate, and dissolve upon the paper with hot, rather dilute nitric acid (1 volume of concentrated acid and 2 volumes of water), passing the acid through the paper several times. Evaporate this solution to dryness, to remove excess of acid. Dissolve the residue in water and test for silver, lead and barium. Heat the entire solution to boiling, and precipitate silver with dilute hydrochloric acid. Filter, to remove silver chloride, and pass hydrogen sulphide through the filtrate to precipitate lead. To test for barium in the filtrate from lead sulphide, first boil to expel hydrogen sulphide, and filter to remove insoluble matter. Then add dilute sulphuric acid, which will precipitate barium sulphate.

For the further identification of silver, dry the hydrochloric acid precipitate and fuse in a porcelain crucible with a small quantity of potassium cyanide. Extract the melt with hot water. Metallic silver will remain undissolved.

The presence of lead may be confirmed by dissolving the hydrogen sulphide precipitate in rather dilute nitric acid and evaporating the solution nearly to dryness. Dissolve the residue in water, and test the solution for lead with sulphuric acid and with potassium chromate.

Barium may be further identified by collecting the sulphuric acid precipitate upon a filter. Thoroughly wash this precipitate, and test in a non-luminous flame upon platinum wire. Barium will impart a yellowish-green color to the flame. This flame may be examined with the spectroscope.

SYNOPSIS OF GROUP III.

The residue, remaining after removal of volatile poisons by distillation, or a portion of original material, is heated upon the water-bath with hydrochloric acid and potassium chlorate, and frequently shaken or stirred. When most of the material has dissolved, and the color of the solution is yellow, dilute with water. Add a few drops of sulphuric acid, and filter the solution when cold.

<table>
<tr><td colspan="4">Material. Treated with HCl and $KClO_3$. Dilute H_2SO_4. Filter.</td></tr>
<tr><td colspan="3">Filtrate. Saturated with H_2S.</td><td rowspan="3">Residue. Tested for "Metallic Poisons IV."
Ag, Pb, Ba.</td></tr>
<tr><td colspan="2">Precipitate. Treated with hot $(H_4N)_2S_x$ and $(H_4N)OH$.</td><td rowspan="2">Filtrate. Tested for "Metallic Poisons III."
Cr, Zn.</td></tr>
<tr><td>Filtrate. Tested for "Metallic Poisons I."
As, Sb, Sn, Cu.</td><td>Residue. Tested for "Metallic Poisons II."
Hg, Pb, Bi, Cu, Cd.</td></tr>
</table>

BIOLOGICAL DETECTION OF ARSENIC BY PENICILLIUM BREVICAULE.

B. Gosio[1] was the first to show that certain moulds, grown upon media containing minute quantities of arsenic, produce volatile arsenic compounds characterized by a garlic-like odor. Seven species of moulds were found to have this power. Penicillium brevicaule, however, which Gosio isolated from air, and which was first found upon decaying paper, possessed this property in the highest degree. Gosio states that we are justified in regarding Penicillium brevicaule as a living reagent for arsenic. Even 0.00001 grams of arsenic can be recognized with certainty by this biological test. The test is so delicate that it should be of great value in toxicological analysis in the preliminary examination for arsenic.

A. Maassen[2] states that a temperature of 28 to 32° is most favorable to the growth of the mould. Crumbs of wheaten bread were found to make an especially good culture-medium. When this material is used, a vigorous growth of mould is visible even in 48 hours. Sometimes a test for arsenic can be finished in a few hours, and always in 2 or 3 days. The characteristic garlic odor from weak, arsenical cultures can be distinctly recognized even after several months. That these "arsenic moulds" do not produce gases having a garlic odor from sulphur, phosphorus, antimony, boron and bismuth compounds, is an important fact. But Penicillium brevicaule possesses in high degree the power of converting solid selenium and tellurium compounds into volatile substances having a peculiar odor. The odor, especially from tellurium cultures, is like that produced by arsenic cultures, namely, distinctly like garlic! The odor from selenium cultures, however, differs from that arising from arsenic cultures. It is more of a mercaptan odor.

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[1] "Azione di alcune muffe sui composti fissi d'arsenico," *Rivista d'igiene è sanità publica*, 1892, 201.

[2] *Arbeiten aus dem Kaiserlichen Gesundheitsamt*, 1902, 478.

Biginelli[1] found that the gases, generated from arsenic cultures by Penicillium brevicaule, are completely absorbed by mercuric chloride solution. Colorless crystals, having the composition $(AsH(C_2H_5)_2.2HgCl_2)$, are formed. This is a double compound of mercuric chloride and diethyl arsine. This compound can easily be decomposed. It then diffuses an intense garlic odor.

R. Abel and J. Buttenberg[2] state that a mould to be of use in the biological detection of arsenic must satisfy the following conditions: "It must grow rapidly, and not generate any odors during growth, except the garlic odor produced from an arsenical medium. It must not be restricted as to culture-medium. It must grow in presence of large, or very small quantities of arsenic. Finally, it must demonstrate its specific action in presence of metallic arsenic and all kinds of arsenical compounds."

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[1] *Chemisches Centralblatt* (1900), II, 1067, and also (1900) II, 1100.

[2] *Zeitschrift für Hygiene*, 32, 440 (1899).

The best material for these experiments is white or Graham bread, either of which is a favorite culture-medium for moulds. The crust is the only part of bread having a specific aromatic odor. When this has been removed, the crumbs may be said to be practically odorless.

Procedure.—When the material being examined is liquid, absorb it completely by adding bread-crumbs, and scatter a small quantity of dry bread over the surface. Solid material should be finely ground, or cut into as small pieces as possible, and placed in not too small a flask. Add at least the same quantity of bread-crumbs, thoroughly mix the two substances by shaking, and moisten the mass with a small quantity of water. Close the flask with a cotton plug, and sterilize in steam. Sterilization must kill all micro-organisms in the flask. Therefore, heat the flask in an autoclave 10 to 30 minutes under a pressure of 1 to 1.5 atmospheres. There is no danger of volatizing arsenic during sterilization. Then inoculate the sterilized material when cold. Place in a flask a slice of potato, superficially coated with mould in the spore-forming stage, and agitate it with bouillon (peptone), salt solution or sterilized water, until it is finely disintegrated. Observe all necessary precautions, and add the mould, suspended in water, in sufficient quantity to impregnate the entire surface of the material suspected of containing arsenic. There should not be more liquid, however, than the culture-medium will absorb. Too much moisture retards the growth of the mould. Finally, draw a tight rubber cap over the mouth of the flask and cotton plug. Flasks thus closed may stand in the room, but it is better to keep them at a higher temperature, for example, in an incubator at 37°, since these conditions are most favorable to the growth of the mould. As soon as a growth of mould is distinctly visible to the naked eye upon the medium, the first indication is given that a test of the culture for volatile arsenic compounds may prove successful. In a very favorable case, this is possible in 24 hours. There is always a luxuriant growth of mould in 48 to 72 hours, so that a decision can be reached. If there be no odor, the flask is closed, and the test is repeated once or twice daily on the following days.

Sulphuric, hydrochloric and other strong mineral acids prevent the growth of the mould. This preventive action may be overcome by neutralization with calcium carbonate, which may be present in excess without ill effect. Alkalies also interfere with the growth of the mould. They may be removed by neutralization with tartaric or citric acid, either of which may be present in excess. The great advantage of the biological over the purely chemical method lies in the fact that less time is required to get a result. The tedious and unavoidable destruction of organic matter in the material is rendered unnecessary. Moreover, a number of tests for arsenic may be made at the same time.

Abel and Buttenberg (loc. cit.) speak regarding this method as follows: "The biological method of detecting arsenic has so many advantages, that it deserves to be recommended for the most varied purposes. Its application is very general, and the method of procedure is simple. The culture of the mould can be kept a long time, even a year or more, without being revived. The test is very delicate, and the odor is readily recognized. The generation of the odor, in the case of cultures containing only 0.0001 grams of arsenic, can be demonstrated for a week."

Besides being practically unlimited in application, the biological method is extraordinarily delicate. In this respect, it exceeds the best known chemical methods for detecting arsenic. It is, for example, considerably more delicate than Bettendorff's test, and it might equal in delicacy the Marsh and Gutzeit tests.

FATE, DISTRIBUTION AND ELIMINATION OF METALS IN THE ANIMAL (HUMAN) BODY.

Arsenic.—Elimination of arsenic takes place mainly through the urine. It begins several hours (7 to 12) after administration, and, in the case of a single dose, usually lasts 4 to 7 days. The length of time arsenic continues to be eliminated in urine, after the last administration has been determined by a large number of experiments, and found to vary from a few days to several weeks. Some authors state that they have found arsenic in urine 80, and even 90, days after poisoning.

As regards retention of arsenic by different organs, it has been observed that large quantities of the poison are always found in the liver. Moreover, the stomach and intestine, with their contents, should be examined, since most of the poison will obviously be in these organs, in case of recent administration. Arsenic is usually found also in the spleen, kidneys and muscles. But the brain rarely contains more than traces of the poison. On the other hand, there are many instances where arsenic has been found in the bones. This fact has given rise to the hypothesis that arsenic acid can replace phosphoric acid in the bones. In arsenic poisoning, depending upon the dose of the drug, the urine is diminished in quantity and usually contains albumin and blood corpuscles.

Arsenic, a Normal Constituent of the Human Body.—Armand Gautier[1] states that he has found traces of arsenic occurring normally in the thymus gland and in the brain of man and of all carnivorous and herbivorous animals. It is said to be present in the thyroid body (glandula thyreoidea) in such quantity that Gautier obtained 1 milligram of arsenic in 127 grams of this gland. Schlagdenhauffen and Pagel found arsenic in the thymus glands of several young animals, and also in human beings, bulls, cows and horses (testicles and ovaries).

Antimony.—Elimination of antimony takes place in great measure through the urine. In antimony poisoning, the metal has been found in the liver, bile and kidneys. A part of the poison is apparently retained in the body for a long time, since antimony has been detected in the liver months after the last administration.

Lead.—Lead is eliminated in urine and faeces. In chronic lead poisoning, elimination by the faeces is said always to exceed that by the urine. Lehmann[2] states that lead is organically combined in urine, that is to say, in such a condition that it cannot be directly precipitated by hydrogen sulphide. Consequently, before tests can be made for lead, the urine must first be heated with hydrochloric acid and potassium chlorate, to destroy organic compounds.

Lead is eliminated especially by the bile, and in acute poisoning this secretion may contain more lead than any of the other organs or secretions. The liver and bile contain a great abundance of lead, when a compound of this metal is administered quickly and in large quantity. But when poisoning goes on more slowly, the liver is said to contain less of the metal, and the kidneys an increased quantity. Oliver[3] gives a case where the yield of lead in milligrams per kilogram of human organ was: liver 41.6; spleen 39.0; large brain 21.6; small brain 8.6; kidneys 13; heart 0.5. Elimination of lead by the urine, in the case of man, is said to be not always uniform. The urine will be free from lead for a long time, and later, without further administration, it will again contain the metal. Lead is present in the faeces partly as sulphide. In chronic lead poisoning, elimination of the metal is always more abundant by the faeces than by the urine. A notable fact about lead poisoning, especially when chronic, is that the brain usually contains the metal in large quantity.

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[1] "Bericht über den internationalen Kongress für Pharmacie in Paris," 1900; also *Pharmaceutische Centralhalle*, 41, 502.

[2] *Zeitschrift für physiologische Chemie*, 6, 6 (1882).

[3] *The Lancet*, March, 1891.

Chromium.—Chromic acid and all soluble chromates and dichromates are quite active poisons. "The alkaline chromates are rapidly absorbed by the mucous membranes, and they produce severe, acute poisoning. The poison manifests itself by causing intense pain in the stomach and intestines, by collapse and kidney derangement, which may terminate fatally in a few hours. Other symptoms noted are nausea, vomiting of yellow matter which later is tinged with blood, diarrhoea and even bloody stools, intense thirst, emaciation, great anxiety, severe pain in the stomach, faint and quickened pulse—the picture presented by a patient suffering from cholera." (Kunkel, Toxikologie.) Statements as to the quantity of an alkaline chromate, which can produce acute poisoning, are fairly concordant. Even a few decigrams (0.2 grams) may cause very serious symptoms, which under certain circumstances may prove fatal. Elimination of chromic acid is mainly by the urine, and partly also by the intestine. It takes place very rapidly, and the body is soon free from the poison. Four days after administration of quite large quantities of chromium, the urine and faeces are said to contain only traces of this metal.

The compounds of chromic oxide (Cr_2O_3), such as chromic hydroxide ($Cr(OH)_3$), chrome alum and chromic sulphate are far less poisonous than chromic acid and its salts. Some authors speak of these compounds even as non-poisonous.

Copper.—The quantity of copper absorbed by the intestine and carried into the circulation is small, and it varies with different copper compounds. Sodium cupric tartrate and the copper salts of the fatty acids are absorbed most easily. It is rare for copper poisoning to occur by the introduction of a copper compound into the stomach. Large doses of copper compounds act as local caustics and occasion severe pain in the stomach. Vomiting and the sense of taste prevent one from taking much of a copper compound. Foods containing copper compounds are unpalatable. The direct sense of taste, as well as the after-taste, makes it impossible to swallow such food in any large quantity. Food, containing 0.5 grams of copper per kilogram, has a marked taste. An irresistible feeling of nausea, which steadily increases, soon makes it impossible to take more of the food contaminated with copper.

Elimination of copper by the urine is very slight. Copper, absorbed in the intestine, is arrested by the liver, where it accumulates. Traces of copper have frequently been found in the human liver. In the course of toxicological analyses, the author has repeatedly found even weighable quantities of copper in livers of adults who had not taken copper salts beforehand, except possibly for suicidal purposes.

Mercury.—The distribution of mercury in the body is said to be always the same, no matter what be the method of administration. It is immaterial, whether it be introduced by the mouth, hypodermically or by an abrasion. The kidneys always contain most mercury, then follow the liver and spleen. The other organs, even the bile, contain at most only traces of mercury. Blood has yielded 0.22 milligrams of mercury per 100 grams. In mercury poisoning the metal can easily be found in the urine, although always present only in small quantity, that is to say, fractions of a milligram in a liter of urine. Elimination of mercury usually continues a very long time after administration. Traces of mercury may be found in the urine for months. Mercury compounds, used in therapeutic doses, are eliminated chiefly by the faeces. Mercury is always eliminated more irregularly and in smaller quantity through the urine than through the faeces. In severe mercury poisoning, the metal appears in all organs and secretions.

Electrolytic Separation of Mercury from Urine.—Heat 1 liter of urine upon the water-bath 2 or 3 hours with 5 or 6 grams of potassium chlorate and 10 c.c. of concentrated hydrochloric acid, and shake frequently. Concentrate to 300 c.c., and use this solution for the electrolytic separation of mercury. Employ a Bunsen battery of 3 or 4 cells, or any other galvanic apparatus having the same strength of current. Use a thin sheet, or rod of gold 2 mm. thick and 6 to 10 cm. long, for the cathode. A piece of platinum wire of about the same size serves as the anode. Usually the electrodes are placed in the solution 2 to 4 cm. apart, and the experiment is allowed to run 24 to 48 hours. The gold cathode, upon which mercury is deposited, is washed, dried and placed in a glass tube (20 cm. long and 4 or 5 cm. in diameter). This tube is sealed at the bottom and reduced at the top to a smaller size. Heat is applied, until all mercury has been expelled from the gold. Care should be taken to deposit the sublimate 3 or 4 cm. beyond the top of the gold rod. Then seal the tube below the sublimate. Introduce a small crystal of iodine into the tube, and seal the other end. If iodine be carefully heated over a small flame, and brought into contact with the mercury, the two elements will combine to form red mercuric iodide.

Mercury can be precipitated from urine upon other metals, for example, copper, gold, brass and zinc dust. Witz destroys organic matter by boiling with hydrochloric acid and a concentrated potassium permanganate solution. Use 10 c.c. of concentrated hydrochloric acid, and 15 to 20 c.c. of potassium permanganate solution for 500 c.c. of urine. Pass the decolorized liquid very slowly through a glass tube over a copper spiral. Wash the copper with potassium hydroxide solution, then with absolute alcohol and cleanse with filter-paper. Finally, dry at 70 to 80° and heat in a glass tube. Treat the mercury sublimate with iodine, as already described. Mercury can be satisfactorily deposited upon other metals (gold, copper), only when organic matter in the urine has previously been destroyed with hydrochloric acid and potassium chlorate. Otherwise, organic substances, deposited upon the metal, interfere with the iodine test for mercury.

Silver.—In severe poisoning, silver has been found in urine, bile, faeces and in many organs. Absorbed silver salts appear to be reduced in all parts of the body. Thus, metallic silver is precipitated in the organs, as has been determined by examining the bodies of persons who have suffered from argyria (chronic silver poisoning). Silver salts, added to albumin solutions, form exceedingly stable compounds which are usually amorphous. Silver may evince even a greater affinity for albumin than for chlorine. All authors agree in saying that only a very small quantity of the silver, which reaches the intestine, is absorbed. After administration of silver, the stools are black, owing to silver sulphide. Silver has been repeatedly determined quantitatively in argyrotic organs. In one case, 0.047 per cent. of silver was found in the liver and 0.061 per cent. in the kidneys. In the condition called argyria, or argyrosis, the skin is gray or black. This color develops gradually, when silver salts are administered internally, and by degrees may become very marked, even causing disfigurement.

Quantitative Determination of Silver in Organs and Urine.—V. Lehmann[1] states that the best and practically the only method applicable to the determination of silver in organs (liver, kidneys, spleen, brain) consists first in thoroughly drying and finely grinding the organ. Then fuse with sodium carbonate and potassium nitrate. Metallic silver is thus obtained. Wash the insoluble residue (silver), which remains after extraction with water, and dissolve in hot nitric acid. Evaporate this solution to dryness upon the water-bath, and precipitate silver as chloride. Avoid a large excess of hydrochloric acid.

Urine should be mixed with sodium carbonate and potassium nitrate, and evaporated to dryness. Fuse the residue, and treat the melt as described.

Zinc.—Without doubt only very small quantities of zinc salts, reaching the intestinal tract, are absorbed. The fate of absorbed zinc has not yet been satisfactorily explained. In zinc poisoning, large quantities of the metal have repeatedly been found in the liver and bile. This may mean that zinc is arrested by the liver, and eliminated with the bile. In an experiment made by Lehmann,[1] a dog, fed upon zinc carbonate for a considerable time, died after 335 days. The following organs were found to contain zinc, and they are arranged according to the quantity of metal in each: liver, bile, great gut, thyroid gland, spleen, pancreas, urine, kidneys, bladder, muscle, brain, lymphatic gland, stomach, small intestine, lungs, blood. Occasionally, very considerable quantities of zinc may be administered with articles of food and drink. Metallic zinc is very freely dissolved by all acids, even by water containing carbon dioxide. Consequently, it may enter drinking water from galvanized pipes, and all kinds of food and drink kept in zinc vessels or vessels coated with zinc. Moreover, plants grown upon soil containing zinc have been found to take up the metal. Zinc has also been found repeatedly in parts of human cadavers under circumstances which precluded all possibility of poisoning by this metal. Even considerable quantities of zinc have been found in the human liver.

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[1] *Zeitschrift für physiologische Chemie,* 6, 19 (1882).

Tin.—The cases of poisoning by tin compounds, thus far observed, resemble those of copper and zinc. Nothing definite, beyond what has been learned from experiments upon animals, is known as to the poisonous action of absorbed tin. These experiments show that small quantities of tin are absorbed and eliminated with the urine, when the ordinary tin compounds are introduced into the stomach. But, thus far, distinct symptoms of poisoning by these quantities of the metal have not been confirmed. (Kunkel, Toxikologie.)

White[2] was unable to produce poisoning by administering tin to a dog by the stomach. The animal took sodium stannous tartrate for 22 days in increasing doses, the daily quantity being 0.02 to 0.06 grams. Yet tin was absorbed by the animal. White found in the urine, collected during an experiment lasting 8 days, 0.02 grams of tin. On the other hand, the tin salt mentioned, when introduced directly into the circulation of the animal, had quite a vigorous, toxic action. Stannous chloride, administered to a dog for a very long time, produced symptoms of poisoning. In this case, the urine contained small quantities of tin. Kunkel (Toxikologie) states that tin has a very slight poisonous action. It seems to be eliminated very quickly by the kidneys, and it is quite likely that this prevents accumulation of the metal in the body and consequent poisoning. White's results, namely, that no symptoms of poisoning appeared, after feeding a dog for 22 days with relatively large quantities of the easily absorbable sodium stannous tartrate; and the fact that Ungar and Bodlander[3] could not produce derangements with the same compound, until it had been administered for a year, prove that tin is quite free from toxic properties. Hence, tin vessels may be used, and preserved articles of food which contain tin have practically no deleterious action upon the health.

[1] *Archiv für Hygiene,* 28, 291 (1896).

[2] *Archiv für experimentelle Pathologie und Pharmakologie,* 13.

[3] *Zeitschrift für Hygiene,* 2, 241.

CHAPTER IV.

POISONS NOT IN THE THREE MAIN GROUPS.

MINERAL ACIDS.

Hydrochloric, Nitric and Sulphuric Acids.

To detect the presence of a free, mineral acid, extract a portion of material with cold water, filter and make the following tests, when the solution has a strong acid reaction:

1. **Methyl Violet Test.**—Add a few drops of an alcoholic solution of methyl violet (1 : 100) to a small portion of solution. A free, mineral acid will produce a blue color.

2. **Congo Paper Test.**—Even very dilute solutions of free, mineral acids turn "Congo paper" blue. Acetic, butyric, lactic and other organic acids have no action upon this paper.

3. **Günzburg's Test.**—Mix a few drops of the filtrate with 3 or 4 drops of Günzburg's reagent and evaporate to complete dryness upon the water-bath, or over a small flame. Free hydrochloric, or sulphuric acid, will give an intensely red or reddish-yellow residue. Nitric acid will give more of a yellowish-red residue.

4. **Sulphocyanate Test.**—Add a small quantity of potassium sulphocyanate solution to ferric acetate solution, and dilute with water, until the color is yellow. Then add the solution to be tested. A free, mineral acid will produce a blood-red color. Traces of free, mineral acid, especially if considerably diluted, will not produce a red color, until several minutes have elapsed.

One or more of these general tests, which furnish evidence of the presence of a free, mineral acid, must always accompany the special tests to be described later. The special tests are given not only by free, mineral acids, but also by their salts in certain cases, and chlorides, sulphates and nitrates are normal constituents of almost all vegetable and animal materials. Parts of cadavers are, as a rule, examined for mineral acids only when the autopsy points conclusively to such poisoning. That is to say, when characteristic corrosions and discolorations are visible, especially about the face, mouth, oesophagus and stomach. When general tests have shown the presence of a free, mineral acid, special tests for the particular acid must be made.

Hydrochloric Acid.

The presence of hydrochloric acid may be recognized by the following tests:

1. **Chlorine Test.**—Warm a small portion of the aqueous extract, which should not be too dilute, with finely powdered black oxide of manganese. Free hydrochloric acid will yield chlorine. It may be recognized by its color and odor, or by passing the gas into potassium iodide solution. This test is not given exclusively by hydrochloric acid. A chloride ($NaCl$) and free sulphuric acid will give chlorine under the same conditions.

2. **Distillation.**—Hydrochloric acid should be separated from other substances by distillation, whenever this is possible. An aqueous extract of the material may be used for this purpose.

In distilling hydrochloric acid, attention must be paid especially to the concentration of the acid. Distillation of dilute hydrochloric acid yields at first nothing but water. Hydrochloric acid[1] itself does not begin to distil with steam, until its strength is about 10 per cent. Since a dilute hydrochloric acid is usually examined, it is necessary to distil almost to dryness the material mixed with water, or preferably a filtered aqueous extract. Heat should be applied in such a distillation by means of an oil-bath. Hydrochloric acid may be detected in the distillate by acidifying with dilute nitric acid, and adding silver nitrate solution. Frequently, hydrochloric acid has to be determined quantitatively. When other acids are absent, the distillate may be titrated with N/10 potassium hydroxide solution, with phenolphthaleïn as indicator. Otherwise, determine the acid gravimetrically by precipitating with silver nitrate, and weighing silver chloride, or volumetrically by Volhard's method. In the latter method, excess of N/10 silver nitrate solution is used in precipitating hydrochloric acid, and the excess is subsequently determined by titration with N/10 ammonium sulphocyanate solution, with ferric alum as indicator. Since the human stomach normally contains from 0.1 to 0.6 per cent. of free hydrochloric acid, an examination of stomach contents for this acid must always include a quantitative determination.

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[1] In the distillation of 100 c.c. of 1 per cent. hydrochloric acid, the first 90 c.c. of distillate will contain only traces of hydrochloric acid, whereas the last portion will contain almost all the acid.

NITRIC ACID.

Nitrates normally occur in the human body only in very small quantity, and they are derived from vegetable foods. Vegetable materials usually contain small quantities of nitrates. Traces of nitric acid can always be found in human urine. In a chemical examination of parts of a cadaver, tests for nitric acid need not be made, unless the autopsy affords evidence of poisoning by this acid. Usually there are distinct signs of corrosion, especially about the lips, mouth, oesophagus and stomach. These parts have a more or less intense yellow or yellowish-brown color. Moreover, the stomach contents, as a rule, also have a yellow color in nitric acid poisoning.

Detection.—Extract the material, when it has a strong acid reaction (see hydrochloric acid), with cold water, and examine the filtrate for nitric acid.

When the quantity of nitric acid is large, it should be separated from other substances by distillation. Nitric acid,[1] however, will not distil with steam, until it reaches a definite strength. At the same time, a large part of the acid will combine with organic substances, if present, forming nitro-derivatives, xanthoproteic acid, etc. It may be expended also in causing oxidation, so that not all the nitric acid originally present will be recovered in the distillate. The residue, after such a distillation, usually has a distinct yellow color. Brown vapors of nitrogen peroxide often appear toward the end of distillation. Such a distillate will cause an immediate blue color, when added to starch paste and potassium iodide, in presence of dilute sulphuric acid. Prepare a filtered, aqueous extract of the material, and distil nearly to dryness from a bath of oil, or calcium chloride.

Nitric acid may be recognized by the following tests:

1. **Diphenylamine and Sulphuric Acid Test.**—Nitric acid produces a blue color with diphenylamine and sulphuric acid. This may be made a zone-test by adding a few drops of diphenylamine sulphate[2] solution to the aqueous extract, or distillate, and carefully pouring this mixture upon pure, concentrated sulphuric acid. There will be a blue zone, where the two liquids meet.

2. **Brucine and Sulphuric Acid Test.**—Nitric acid produces a red color with brucine and sulphuric acid. This may be made a zone-test by mixing the liquid with the same

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[1] If 100 c.c. of 1 per cent. nitric acid be mixed with bread-crumbs and distilled, the greater part of the acid will be in the final 10 c.c. of distillate.

[2] This solution is prepared by dissolving 1 gram of diphenylamine, $(C_6H_5)_2NH$, in 5 grams of dilute sulphuric acid and 100 grams of water.

volume of sulphate of brucine[1] solution, and carefully pouring this mixture upon pure, concentrated sulphuric acid. There will be a red zone, where the two liquids meet.

3. **Ferrous Sulphate and Sulphuric Acid Test.**—Saturate the liquid to be tested with pure ferrous sulphate, and carefully pour this solution upon pure, concentrated sulphuric acid. There will be a black zone, where the two liquids meet.

4. **Copper Test.**—The action of nitric acid upon a small piece of clean copper (wire or sheet) is especially characteristic. Heat will produce red-brown vapors of nitrogen peroxide (NO_2). Very dilute nitric acid will not give this test. The filtrate from the aqueous extract of the material, if too dilute, cannot be used for this test.

SULPHURIC ACID.

Animal and vegetable materials usually contain traces of sulphates. Furthermore, nearly all animal and vegetable liquids, juices and ashes normally contain sulphates. Therefore, there must always be proof that free sulphuric acid is present. Extract the material, if it have a strong acid reaction, with cold, absolute alcohol and filter after some time. Sulphuric acid will be dissolved, but not the sulphates of the alkalies and of the alkaline earths. Evaporate the alcoholic filtrate upon the water-bath, and dissolve the residue in a small quantity of water. Boil the solution to saponify ethyl-sulphuric acid, and, after filtering, test the strongly acid liquid for sulphuric acid with barium chloride, or lead acetate solution. To obtain further proof that the precipitate is a sulphate, mix with sodium carbonate and fuse upon charcoal. This treatment will produce sodium sulphide which, in presence of water, will blacken metallic silver, or give hydrogen sulphide with acids. The material may also be extracted with water, and the following tests for free sulphuric acid may be made with the filtrate:

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[1] This solution is prepared by dissolving 1 gram of brucine in 5 grams of dilute sulphuric acid and 100 grams of water. The sulphuric acid, used in this test, must give none of the tests for nitric acid. Should the sulphuric acid not meet these requirements, it must be heated in a platinum dish, until the interfering nitrous-substances have been removed; or the acid may be distilled from a small retort, and the first part of the distillate may be rejected.

1. **Sugar Test.**—Evaporate a small portion of the filtered extract upon a small particle of sugar. When free sulphuric acid is present, there will be a black, carbonaceous residue.

2. **Sulphur Dioxide Test.**—Concentrate the filtered aqueous extract upon the water-bath, and heat with a few pieces of copper. If the material contain free sulphuric acid, sulphur dioxide will be generated. Distil the sulphur dioxide into a small quantity of water. Add stannous chloride solution to a portion of the distillate. Sulphur dioxide will act as an oxidizing agent upon the stannous salt.[1] If iodine solution be used instead of stannous chloride, it will be decolorized, and sulphur dioxide will be oxidized to sulphuric acid.

Obviously, in examining parts of a cadaver, there is no need of testing for sulphuric acid, unless the autopsy point conclusively to poisoning by this acid. That is to say, when the mouth, oesophagus and stomach are considerably corroded and discolored. In such a case, the stomach externally will be colored partly brown, and partly slate-gray, and usually the stomach-contents will have a blackish color.

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[1] Sulphurous acid and sodium sulphite precipitate from stannous chloride solution not too strongly acid, stannous sulphite, $SnSO_3$, white, readily soluble in HCl. When warmed in the presence of hydrochloric acid, sulphur dioxide acts as an oxidixing agent upon a stannous salt. A precipitate of $Sn_6O_{10}S_2$ or SnS_2 is formed, or H_2S is evolved and $SnCl_4$ formed, depending upon the amount of HCl present. (Prescott and Johnson, Qualitative Chemical Analysis. Fifth edition, page 86.) Tr.

Sulphuric acid is determined quantitatively by titration with N/10 potassium hydroxide solution, each c.c. of decinormal alkaline solution being equivalent to 0.0049 grams of sulphuric acid. This acid may be determined quantitatively also by precipitating and weighing barium sulphate.

DETECTION OF FREE ALKALIES.

Potassium, Sodium and Ammonium Hydroxides.

Free Alkalies.—The same general principles, used in detecting mineral acids, are applicable also to the alkalies. Since potassium and sodium compounds are normal constituents of animal and vegetable organisms, and since ammonia is a decomposition product of nitrogenous, organic matter, the examination must always show that the alkalies are in the free state. It is only when they are in this condition that they decompose and corrode animal tissues.

Procedure.—When the material has a strong alkaline reaction, extract several times with absolute alcohol. Use a flask having a glass stopper, and distil the combined extracts.

Free ammonia will be in the distillate, and the customary tests for its identification may be made with this solution.

Potassium and sodium hydroxides will be in the residue. When the residue is strongly alkaline, add first a few drops of phenolphthaleïn solution and then excess of barium chloride. If alkaline carbonates have caused the red color of the phenolphthaleïn, the alkaline reaction will disappear.

$$K_2CO_3 + BaCl_2 = BaCO_3 + 2KCl.$$

At the same time, the color of the solution will be discharged. On the contrary, if alkaline hydroxides be present, both alkaline reaction and red color will remain.

$$2KOH + BaCl_2 = Ba(OH)_2 + 2KCl.$$

To distinguish between potassium and sodium hydroxides, dissolve the residue in a small quantity of water, and test for potassium with chloroplatinic acid solution, or with solution of sodium cobaltic nitrite, $6NaNO_2.Co_2(NO_2)_6$, (de Konink's reagent[1]), which produces a yellow precipitate of potassium cobaltic nitrite, $6KNO_2.\ Co_2(NO_2)_6 + xH_2O$, when added to a solution containing a potassium salt. To hasten the reaction, add a few drops of acetic acid. Test for sodium in a neutral solution by adding a few drops of freshly prepared acid potassium pyro-antimonate solution, $K_2H_2Sb_2O_7$. At first this produces only a turbidity but, on stirring, a white, crystalline precipitate of acid sodium pyro-antimonate, $Na_2H_2Sb_2O_7$, is deposited.

In Vitali's method, the alcoholic extract of the material is shaken with freshly precipitated and well-washed mercurous chloride. Free alkali will blacken this compound. Mercurous oxide (Hg_2O), the black compound formed, can be distinguished from mercuric sulphide by its solubility in dilute nitric acid.

Quantitative Determination of Alkaline Hydroxides.—It is customary to determine both the caustic alkali present in the free condition, and that which has been converted into carbonate. First, determine total alkalinity by titrating a portion of the residue. Then precipitate carbonate in a second portion with barium chloride, and determine free caustic alkali in the filtrate.

Ammonia.—Determine free and combined ammonia in the distillate before and after addition of magnesium oxide.

POTASSIUM CHLORATE.

Potassium chlorate ($KClO_3$) in large doses has a decidedly poisonous action. It changes the red corpuscles, and converts oxyhaemoglobin into methaemoglobin. The red corpuscles alter their shape and are decomposed. Potassium chlorate is quite rapidly eliminated from the living organism by the kidneys. After administration of 0.1 gram of potassium chlorate, this substance can be detected in the urine by the indigo test after an hour. Most of the potassium chlorate passes unchanged into the urine. Marchand and E. Schmidt state that potassium chlorate, administered to a dog, can be recovered in the urine almost quantitatively. Consequently, only a small quantity of the salt is reduced to potassium chloride in metabolism. This partial reduction varies according to the quantity of potassium chlorate administered. It is greater with large than with small doses, and in the latter case, under certain circumstances, it can no longer be detected. During poisoning by potassium chlorate, the urine is usually very dark, even black and opaque. A dark brown sediment is gradually deposited. The urine contains considerable albumin and is usually alkaline. When poisoning by potassium chlorate is suspected, the urine should receive a thorough chemical and microscopical examination.

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[1] Sodium cobaltic nitrite solution may be prepared by dissolving 10 grams of pure sodium nitrite and 4 grams of cobaltous nitrate separately in sufficient water. Mix the solutions, add 2 c.c. of acetic acid and dilute to 100 c.c. with water.

Procedure.—Subject parts of the cadaver, for example, the contents of the stomach and intestine, and parts of the various organs finely minced, to dialysis. Evaporate the dialysate to dryness upon the water-bath, and dissolve the residue in a small quantity of water. Filter this solution, and examine the filtrate for chloric acid.

1. **Indigo Test.**—Add dilute sulphuric acid and a few drops of indigo solution, until there is a distinct blue color, to the solution. Then introduce sulphurous acid drop by drop. If chloric acid be present, the blue color will disappear, and change to yellow or greenish-yellow. This is a delicate test for chloric acid, by which even 0.01 grams of potassium chlorate can be detected.

2. **Silver Nitrate Test.**—Add silver nitrate solution in excess to the solution. If there be a precipitate (AgCl), filter and add a few drops of sulphurous acid to the clear filtrate. When a chlorate is present, more silver chloride will be precipitated. Silver chloride differs from silver sulphite in being insoluble in dilute nitric acid. Sulphurous acid reduces a chlorate to a chloride.

3. **Free Chlorine Test.**—When a dialysate, containing a chlorate, is heated with concentrated hydrochloric acid, chlorine is set free. Pass the gas into potassium iodide solution. To detect free iodine, shake the solution with chloroform which will dissolve iodine with a violet color. This test is proof of chloric acid, only in the absence of other substances like chromic acid and dichromates, which also evolve chlorine with hydrochloric acid.

When the substance is a powder, dissolve it in water and, if necessary, filter. Usually such a solution can be tested directly for chloric acid.

Potassium chlorate can easily be determined quantitatively in urine, dialysates and other liquids by reducing it to potassium chloride with zinc dust.

Divide the solution to be examined into two equal parts. Determine chloride gravimetrically in one portion by precipitating and weighing AgCl, or volumetrically by titrating according to Volhard's method.

Determine chloride and chlorate together in the second portion. Add 5 to 10 grams of zinc dust, and a small quantity of dilute sulphuric or acetic acid, and heat this mixture for 0.5 to 1 hour upon a boiling water-bath. Filter, and wash the residue with boiling water. Acidify the filtrate with nitric acid, and precipitate chloride with silver nitrate. Obviously, more chlorine will be found in the second than in the first determination. The percentage of potassium chlorate can easily be calculated from the difference between the two chlorine determinations. One molecule of $KClO_3$ yields upon reduction 1 molecule of KCl.

Behavior of Potassium Chlorate in Putrefaction.—C. Bischoff[1] states that potassium chlorate, mixed with moist, organic substances, especially blood, is very soon reduced to chloride! Bischoff describes several cases, in which poisoning by potassium chlorate had undoubtedly occurred, and yet chloric acid could not be detected chemically in parts of the cadaver.

In an experiment, 100 grams of blood, 0.5 grams of potassium chlorate and 100 grams of water were allowed to stand for 5 days at room temperatures. Not a trace of chloric acid could be detected in the dialysate. C. Bischoff concludes from this experiment that potassium chlorate, mixed with moist, organic substances, especially with blood, is soon reduced. Consequently, chloric acid may not be detected, even in cases of rapidly fatal poisoning by potassium chlorate.

OXALIC ACID.

Oxalic acid and its salts (for example, salt of sorrel, KHC_2O_4) are quite strong poisons which act quickly. Free oxalic acid, like a strong acid, has a very corrosive action. Administration of oxalic acid has caused fatal results in a few minutes. Oxalic acid is very abundant in the vegetable kingdom, and may find access to the body through food and drugs of vegetable origin. Consequently, in toxicological analysis it is often necessary to supplement a positive, qualitative test by a quantitative determination of oxalic acid. Moreover, oxalic acid is a normal constituent of human urine, 2 to 6 milligrams being excreted in the course of a day.

Bischoff[2] has made statements in regard to the distribution of oxalic acid in the different organs of persons poisoned by this substance. In a case, which terminated fatally in less than 15 minutes, the quantity of oxalic acid in each organ was determined separately and found to be:

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[1] *Berichte der Deutschen chemischen Gesellschaft,* 16, 1343 (1883).

[2] *Berichte der Deutschen chemischen Gesellschaft,* 16, 1350 (1883).

Weight.		Organ.	Oxalic Acid.	
2240 grams		Stomach, oesophagus, intestine and contents	2.28	grams.
770	"	Liver	0.285	"
290	"	Kidneys	0.0145	"
180	"	Blood from the heart	0.0435	"
40	"	Urine	0.0076	"

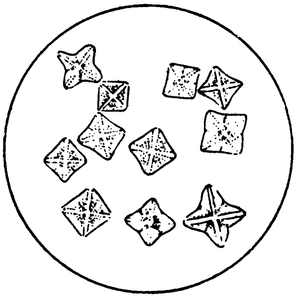
The quantity of oxalic acid in the liver is noticeably large. The kidneys and urine contain only a small quantity of the poison, owing to the short duration of life after poisoning. Urine, excreted during oxalic acid poisoning, deposits crystallized calcium oxalate in great abundance.

When it is sufficient to detect oxalate, without discriminating between the free acid, acid potassium oxalate and calcium oxalate, the following method may be employed:

Add 3 or 4 times the volume of alcohol to the finely divided material, and acidify strongly with dilute hydrochloric acid. Stir frequently, and allow the mixture to digest in the cold for 1 or 2 hours. Then pass the solution through a plaited filter, moistened with alcohol, and wash the residue with alcohol. Add 10 to 15 c.c. of water to the total filtrate, to prevent formation of ethyl oxalate during evaporation. Evaporate to about 10 c.c. upon the water-bath, that is to say, until all alcohol has been expelled. Dilute the residue with about 10 c.c. of water, and pass the solution through a small filter. Make 3 or 4 extractions of the filtrate in a separating funnel with 50 c.c. portions of ether. Pass the total ether extract, after it has stood in a dry flask for some time, through a dry filter and distil. When the quantity of ether is small, evaporate it in a small glass dish. Dissolve the residue in 2 or 3 c.c. of water, and pass the solution, if necessary, through a moist filter. Render alkaline with ammonium hydroxide solution, and add saturated calcium sulphate solution. If there be a precipitate, acidify slightly with acetic acid, and let solution and precipitate stand in a covered beaker over night. If a crystalline precipitate remain, it can be only calcium oxalate. A thorough microscopic examination of this precipitate is always advisable. Calcium oxalate appears in characteristic octahedrons having the so-called envelope-form (Fig. 14). Calcium oxalate, when thoroughly washed, may be converted into calcium oxide by ignition, and this residue may be weighed.

$$CaO : (H_2C_2O_4.2H_2O) = 56 : 126.$$

FIG. 14.



Calcium Oxalate Crystals.

DETECTION OF SANTONIN, SULPHONAL, TRIONAL (ACETANILIDE, PHENACETINE).

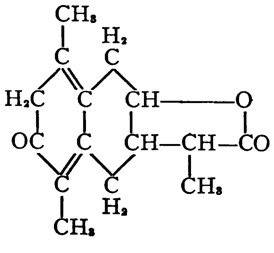
The compounds, considered here, do not find a place in the Stas-Otto process, on account of their behavior as regards solubility in cold water containing tartaric acid and in ether. The following method for detecting these substances may be employed.

Make the material neutral, or faintly acid with tartaric acid, and extract with boiling absolute alcohol under a reflux condenser. Filter, and evaporate the filtrate to dryness upon the water-bath. Dissolve the residue in hot water. If it be colored, or very impure, digest upon the water-bath for some time with bone-black, and shake frequently. Filter the solution while hot. When one of the above substances is present in considerable quantity, a part will crystallize as the solution cools. Thoroughly extract the filtered, aqueous solution and any crystals several times with chloroform. Remove the chloroform extract from the separating funnel, and pass through a dry filter. The residue from chloroform may contain santonin, sulphonal and trional, as well as acetanilide and phenacetine.

Obviously, the chloroform residue will also contain those substances, which are extracted by ether from the acid solution in the Stas-Otto process. Substances like antipyrine, caffeïne, acetanilide, phenacetine and salicylic acid will be extracted completely by chloroform. As a rule, they will be obtained purer than in the usual ether extraction. This chloroform residue may also contain narcotine which is a weak base.

Santonin.

Santonin ($C_{15}H_{18}O_3$) crystallizes in colorless, inodorous, shining leaflets. These crystals are bitter and melt at 170°. Santonin is soluble in 5000 parts of cold water and in 250 parts of boiling water, in 44 parts of alcohol and in 4 parts of chloroform. All these solutions are neutral. It is slightly soluble in ether (1 : 150). Light turns these crystals yellow. An alcoholic solution of this yellow modification, upon evaporation, deposits white santonin. Santonin is the anhydride (lactone) of santonic acid ($C_{15}H_{20}O_4$). Alkalies convert it into salts of this acid. At 120° santonic acid decomposes into santonin and water. The most probable formula of santonin shows that this substance is at the same time a lactone and a ketone. It is apparently a derivative of a hexahydro-dimethyl-naphthalene. Santonic acid, upon fusion with potassium hydroxide, gives dimethyl-β-naphthol in addition to hydrogen and propionic acid.



Santonin may be recognized by the following tests:

1. **Alcoholic Potassium Hydroxide Test.**—Santonin, heated with an alcoholic solution of potassium hydroxide, will give a beautiful carmine-red color. This color changes gradually to reddish-yellow, and

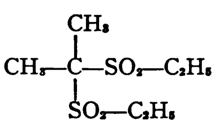
finally fades entirely. In this test yellow santonin at once dissolves with a yellowish-red color.

2. **Sulphuric Acid-Ferric Chloride Test.**—Shake about 0.01 grams of santonin with a cold mixture of 1 c.c. of pure sulphuric acid and 1 c.c. of water. There is almost no color, but a drop of ferric chloride solution, added to the mixture heated almost to boiling, will give a violet color.

3. **Furfurol-Sulphuric Acid Test.**—Mix 2 or 3 drops of an alcoholic solution of santonin with 1 or 2 drops of 2 per cent. alcoholic furfurol solution, and then with 2 c.c. of pure, concentrated sulphuric acid. A splendid purple-red color will appear and change, upon further warming, in succession to crimson-red, blue-violet, dark blue and finally to black. Not many alkaloids and glucosides give distinct color reactions with furfurol and sulphuric acid. Substances behaving similarly are veratrine, picrotoxin (violet), piperine (green to blue-green, finally indigo-blue) and α- and β-naphthol.

Behavior of Santonin in the Body.—Santonin seems to be incompletely absorbed in the body. M. Jaffé[1] has administered quite large quantities of santonin to dogs and rabbits. He obtained a new substance, called α-oxysantonin ($C_{15}H_{18}O_4$), from the urine of the dog, amounting to 5 or 6 per cent. of the santonin administered. He extracted with chloroform considerable quantities of unaltered santonin from the faeces of the dog. Rabbits can usually tolerate being fed with santonin for weeks, and α-oxysantonin is formed only in very small quantity. In the ether extract of the rabbit's urine, Jaffé found a second santonin derivative, β-oxysantonin, isomeric with α-oxysantonin, with considerable unaltered santonin. In these experiments only about half the santonin administered was absorbed by the rabbit.

SULPHONAL.



$$CH_3—\overset{\displaystyle CH_3}{\underset{\displaystyle SO_2—C_2H_5}{C}}—SO_2—C_2H_5$$

Sulphonal crystallizes in colorless, inodorous and tasteless prisms. It melts at 125 to 126°, and distils with slight decomposition at 300°. It is soluble in 500 parts of cold water and in 15 parts of boiling water, in 135 parts of ether and in 65 parts of cold alcohol and 2 parts of boiling alcohol. It is freely soluble in chloroform. Sulphonal is very stable in presence of chemical reagents. The halogens, halogen acids, alkaline hydroxides and carbonates, concentrated sulphuric and nitric acids have no action upon this substance in the cold.

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[1] *Zeitschrift für physiologische Chemie*, 22, 538 (1896/97).

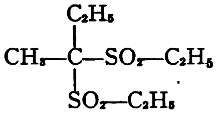
Sulphonal may be recognized by the following tests:

1. **Vulpius' Test.**—Fuse 1 part of sulphonal and about 2 parts of potassium cyanide in a dry test-tube. The characteristic odor of ethyl mercaptan ($C_2H_5—SH$) will be developed, and the aqueous solution of the melt, after acidification with dilute hydrochloric acid, will give a deep red color with 1 or 2 drops of ferric chloride solution. This is the sulphocyanate test. Potassium sulphocyanate is produced when sulphonal is fused with potassium cyanide. This test is given very distinctly by 0.02 grams of sulphonal.

2. **Reduction Test.**—When sulphonal is heated in a test-tube with powdered wood-charcoal, the characteristic odor of ethyl mercaptan is developed.

3. **Melting-Point Test.**—Determination of the melting-point (125 to 126°) should be made, when the substance is perfectly pure.

TRIONAL.



$$\begin{array}{l} \quad C_2H_5 \\ \quad | \\ CH_3—C—SO_2—C_2H_5 \\ \quad | \\ \quad SO_2—C_2H_5 \end{array}$$

Trional crystallizes in colorless, shining leaflets. It melts at 76°. It is soluble in 320 parts of cold water and more easily soluble in hot water. It is also soluble in alcohol and ether. The aqueous solution is neutral and bitter. In the latter respect, it differs from sulphonal, which is tasteless. Trional gives the sulphonal reactions.

Detection of Sulphonal (Trional) in Urine.—Sulphonal[1] can be detected in urine after large doses, especially if administered continuously. Such urine often has a dark-red to garnet-brown color, due to haematoporphyrin. But this decomposition product of haemoglobin appears in urine, only after severe poisoning by sulphonal, and even then its occurrence is rare.

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[1] Sulphonal has a strong cumulative action and, consequently, collects in the body in considerable quantity, when administered continuously in large doses.

To isolate sulphonal from urine, evaporate 1000 c.c. to one-tenth its volume, and extract several times with large quantities of ether. Pass the ether extracts, after they have settled in a dry flask for several hours, through a dry filter and distil. Evaporate the residue with 20 to 30 c.c. of 10 per cent. sodium hydroxide solution to dryness upon the water-bath. This will remove coloring-matter, extracted from urine by ether. Extract sulphonal from the alkaline residue with ether. Evaporate the solvent, and sulphonal will remain pure and almost colorless. Determine the melting-point of this residue, and make the other tests for sulphonal.

Detection of Haematoporphyrin in Urine.—Coloring-matters have been observed in red, brownish-red to cherry-red urines, which quite probably are identical with haematoporphyrin. The spectroscopic examination of such urine is made in the following manner. Add sodium hydroxide solution, drop by drop, to about 500 c.c. of urine, until the reaction is strongly alkaline, and then add a small quantity of barium chloride solution. Filter after a while, and wash the precipitate well. Extract the precipitate upon the filter with hot alcohol, containing a few drops of dilute sulphuric acid. A spectroscopic examination of this filtrate can be made directly with a Browning pocket-spectroscope. Acid haematoporphyrin solutions are violet; when more concentrated, they have a cherry-red color, and show the characteristic spectrum with two absorption-bands. (See page 170.) If the acid, alcoholic solution be saturated with a few drops of ammonium or sodium hydroxide solution, the spectrum of alkaline haematoporphyrin solution with its four absorption-bands will appear. Traces of haematoporphyrin very frequently appear in normal urine. It has been observed more abundantly, at times, in urine during chronic sulphonal poisoning.

EXAMINATION OF BLOOD.

1. DETECTION OF CARBON MONOXIDE IN BLOOD.

Carbon monoxide (CO) has a direct, poisonous action upon the blood. It displaces the loosely bound oxygen of oxyhaemoglobin, when passed into blood, and forms the more stable chemical compound, carboxyhaemoglobin. Carboxyhaemoglobin is cherry-red, not dichroic, and entirely resistant to putrefaction, when air is excluded. The cherry-red color of blood in poisoning by carbon monoxide is usually noticeable at once.

Carbon monoxide in blood may be recognized by the following tests:

1. **Sodium Hydroxide Test.**—A few drops of sodium hydroxide solution, added to blood containing carbon monoxide, will produce more or less of a vermilion color. Dilute the blood with 6 to 10 times its volume of water, and add about 5 drops of sodium hydroxide solution to 10 c.c. of blood solution. Under these conditions, an aqueous solution of normal blood, containing oxyhaemoglobin, will have a green to brownish-black color, especially if heat be applied. On the contrary, the solution of carboxyhaemoglobin, if warmed gently with sodium hydroxide solution (10 per cent. NaOH), will not change its red color.

2. **Potassium Ferrocyanide Test.**—Add the same volume of 20 per cent. potassium ferrocyanide solution, and 2 c.c. of dilute acetic acid (1 volume of glacial acetic acid and 2 volumes of water) to undiluted blood. Shake the mixture gently, and the blood will be gradually coagulated. Normal blood will give a dark brown coagulum, whereas that from blood containing carbon monoxide will be bright red. This difference disappears slowly, but not completely for weeks.

3. **The Tannin Test.**—Add three times the volume of 1 per cent. tannin solution to an aqueous solution of blood (1:4), and shake thoroughly. A difference in color between normal blood and that containing carbon monoxide can be recognized after several hours, most distinctly after 24 hours. The color of normal blood is gray, whereas that of blood containing carbon monoxide is crimson-red. This difference is apparent, even after several months. Ten per cent. of carboxyhaemoglobin can be detected in blood by means of tests 2 and 3.

4. Palladous Chloride Test.—Carbon monoxide produces a black precipitate of metallic palladium with neutral, aqueous solutions of palladous chloride.

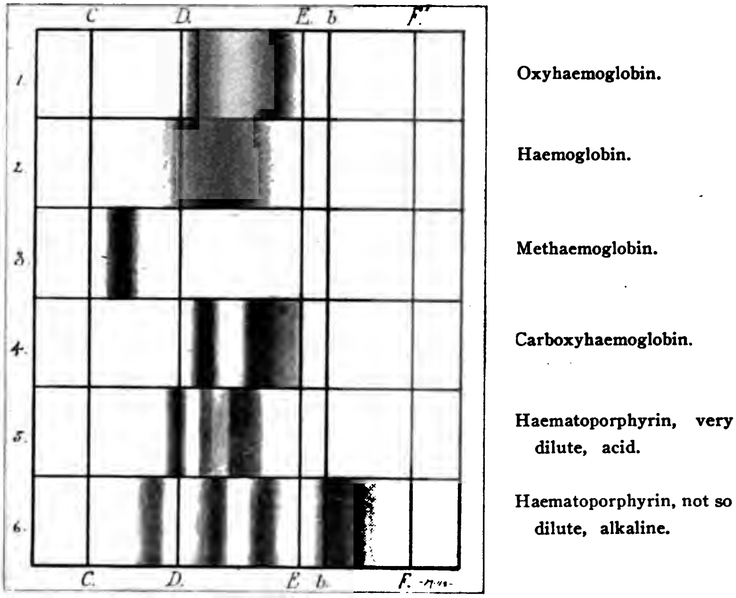
$$CO + H_2O + PdCl_2 = CO_2 + 2HCl + Pd.$$

Add a few drops of potassium hydroxide solution to the blood, and warm gently upon the water-bath. Draw through the solution air, which has been washed until pure, and pass the gas evolved, first through lead acetate solution, to remove possible hydrogen sulphide, then through sulphuric acid, to absorb ammonia, and finally through a neutral, light red palladous chloride solution (1 : 500).

5. Spectroscopic Examination.—It is comparatively easy to detect carboxyhaemoglobin with the spectroscope. The two absorption-bands of this substance are quite similar to those of oxyhaemoglobin, but they are somewhat nearer together and located more toward violet. The principal difference, however, between the two absorption-bands of carboxyhaemoglobin and oxyhaemoglobin is, that those of the former substance are not extinguished by reducing agents. The blood solution, used in a spectroscopic examination, is prepared by diluting 1 to 1.5 parts of blood with 100 parts of water, and the observations are made through a layer 1 cm. thick. One per cent. blood solution may be reduced by adding a few drops of ammonium sulphide solution, mixing thoroughly, and adding 4 to 6 more drops of the same reagent as a surface-layer to exclude air. Reduction begins in 6 to 8 minutes. A solution of tartaric acid and ferrous sulphate, to which an excess of ammonium hydroxide solution has been added, may also be used to reduce oxyhaemoglobin.

Under these conditions, oxyhaemoglobin is changed to reduced haemoglobin. The two absorption-bands, characteristic of the former substance, disappear, and a broad, diffuse absorption-band occupies the previous bright space between the two bands. The spectrum of carboxyhaemoglobin remains unchanged, only when 27 per cent. at least of the haemoglobin is saturated with carbon monoxide. If allowed to stand in an open vessel, blood will

FIG. 15.



Absorption-Spectra.

lose carbon monoxide within 8 days. Blood, containing carbon monoxide and sealed in glass tubes, is said, however, to keep for years. Carbon monoxide has been detected in the blood of a cadaver after 18 months.

B. Tollens[1] recommends adding some formaldehyde to the blood solution. This reagent has no effect upon the two oxyhaemoglobin bands. But, upon warming the mixture very cautiously with ammonium sulphide solution, a third and nearly as distinct black band will appear almost midway between the original bands which gradually disappear. Finally, only this band will remain. This is a far more satisfactory test than is afforded by the indefinite band given by blood alone. If the solution be cooled and agitated with air, this third band will disappear, and the two original oxyhaemoglobin bands will return.

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[1] *Berichte der Deutschen chemischen Gesellschaft*, 34, 1426 (1901).

When carbon monoxide is present, formaldehyde does not have this action.

2. Detection of Blood-Stains.

The detection of blood, when in the form of dry stains upon wearing-apparel, wood, knives, weapons, etc.,[1] is more certain and less open to question, if haemin crystals (Teichmann's blood-crystals, haematin hydrochloride) be prepared from the blood-pigment. If haemin crystals be obtained, the stain in question may be regarded with certainty as having been caused by blood. Fresh, dried blood is bright red and has a smooth surface. Flakes of such blood, scraped from any material, are garnet-red by transmitted light. A solution of fresh blood-stains in potassium or sodium hydroxide is dichroic. The solution is red by transmitted light, and green by reflected light. Later, dried blood becomes brownish-red or brownish-black. These color changes are due to conversion of oxyhaemoglobin, first into methaemoglobin and then into haematin. The first two substances are soluble in water, but the last is not. Haematin, however, is soluble in alkalies, and in alcohol containing sulphuric acid. This change of the blood-pigment depends not upon the age of the stain, so much as upon the action of air (oxygen), light, heat and moisture upon the blood before it is dry. When blood is in small drops, haemoglobin under certain conditions will change into methaemoglobin, even in 3 to 10 days. boiling water causes immediate insolubility. The action proceeds very rapidly also in direct sunlight. Washing in alkaline solutions (boiling solutions of potassium or sodium soap, sodium carbonate solution, ammonia and sewage) also causes speedy decomposition. Acids, on the other hand, nitric and hydrochloric, as well as putrefaction, act more slowly, giving the blood a laked appearance and even making it clear and colorless. But when blood has once dried unchanged, these injurious agencies act less vigorously. Even putrefaction does not proceed so easily.

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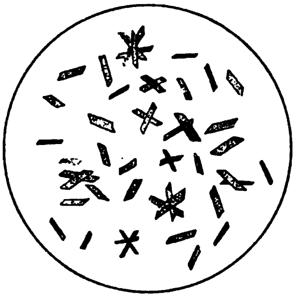
[1] Blood mixed with oxide of iron (for example, blood upon rusty knives and weapons) usually fails to give haemin crystals.

FIG. 16.



Haemin Crystals.

Haemin Crystals (Teichmann's Crystals).—To obtain these crystals, scrape the blood-stains from the material, or extract them with water and evaporate the solution. Warm the dry blood-stain upon a watch-glass, or glass slide, with a few drops of glacial acetic acid and a trace of sodium chloride.[1] Heat the glass upon the water-bath, until there is no odor of acetic acid. Examine the residue with a microscope, magnifying about 300 times. Haemin crystals are brownish-red to brownish-black by transmitted light. They form long rhombic plates which frequently lie crossed upon each other (Fig. 16). If these crystals fail to appear upon the first evaporation, a second treatment with a few drops of glacial acetic acid is necessary. Frequently, only glacial acetic acid will extract the pigment from old blood-stains. Evaporate this solution with a trace of sodium chloride. In Brücke's method the stains, or the scrapings, are heated directly to boiling in a test-tube with 10 to 20 drops of glacial acetic acid. The decanted or filtered solution is evaporated to dryness upon a watch-glass at 40 to 80°, after addition of a trace of sodium chloride. The residue is examined with a microscope. In this method it is immaterial whether the blood has coagulated or not.

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[1] Strzyzowski (*Chemisches Centralblatt,* 1897, I, 295) advises using sodium iodide instead of sodium chloride. Place a small particle of material suspected of containing blood upon a glass slide and add a drop of sodium iodide solution (1:500). Evaporate and cover with a cover-glass. Heat for 3 to 6 seconds with concentrated acetic acid which is allowed to run under the cover-glass. The test with this modification is said to be more delicate, owing to the darker color of the haematin hydriodide crystals. The crystals are usually obtained in less time and with as small a quantity as 0.000025 grams of fresh blood. Tr.

Cold water has no effect upon blood-stains, when an attempt has already been made to remove them with hot water. The proteïn substances in the blood have been coagulated and rendered insoluble in water. In a case of this kind, treat the stain with water containing a few drops of sodium hydroxide solution. When the stains are upon woolen material, very dilute sodium hydroxide solution must be used, because alkalies dissolve wool. Water containing ammonium hydroxide may be used to extract stains, since this alkali has no action upon wool. The alkaline, aqueous extract may be used in preparing haemin crystals. Evaporate the solution in a watch-glass to dryness upon the water-bath, and mix the residue thoroughly with 8 to 10 drops of glacial acetic acid. Add a trace of sodium chloride and again evaporate. After acidifying the extract of the stain with acetic acid, it is often advisable to add tannic acid, or zinc acetate, and to prepare Teichmann's crystals from the precipitate.

Not infrequently, suspected stains have to be extracted with hot alcohol containing sulphuric acid. Haematin, formed from the blood-pigment, is dissolved. The solution has a brown color, when this substance is present. Excess of sodium hydroxide solution will produce the dichroism, characteristic of an alkaline haematin solution, namely, red by transmitted light and green by reflected light. Obviously, haematin should be identified by the spectroscope, both in acid and alkaline solution.

Blood mixed with oxide of iron (for example, blood upon rusty weapons) usually fails to give haemin crystals, although the extract with dilute sodium hydroxide solution frequently shows the dichroism of haematin solution. Since iron oxide, or rust, forms an insoluble compound with haematin, such stains should be warmed for some time with sodium hydroxide solution upon the water-bath, to dissolve haematin which may be present.

Note.—Perfectly. fresh blood-stains may be detected by observing blood-corpuscles with the microscope. Human blood can be differentiated from animal blood by comparing the blood corpuscles as to size with those of animal blood, only when the corpuscles are still intact.

Spectroscopic Detection of Blood. (See pages 169–171.)—If a brownish blood-stain be extracted with water, a third, fainter and narrower band will appear in addition to the two oxyhaemoglobin bands. This lies in the orange between C and D and is the methaemoglobin band.

Acetic acid, provided the oxyhaemoglobin solution be not too dilute, will discharge the two bands. At the same time, the color of the solution will become mahogany-brown, due to formation of haematin in acid solution. This solution has a characteristic spectrum, namely, four absorption-bands in the yellow and green. Excess of ammonium hydroxide gives an alkaline solution of haematin. This may be recognized by a broad, faint absorption-band lying between the red and yellow. A few drops of ammonium sulphide solution will extinguish this band, and bring out two broad bands, namely, one in the green and the other in the light blue. These bands lie farther to the right than those of oxyhaemoglobin and have about the same width. This is the spectrum of reduced haematin (haemochromogen). All these spectroscopic tests are very characteristic, especially the spectra of oxyhaemoglobin, haemoglobin and, in the case of old blood-stains, that of reduced haematin.

Up to the present time, no red solution has been found, which, upon abstraction and addition of oxygen, will give the same spectroscopic phenomena as blood!

When the quantity of blood is very small, or when the blood-pigment has undergone further decomposition, so that the bands of oxyhaemoglobin are no longer visible, it is advisable to extract the stains for several hours with concentrated potassium cyanide solution. Blood will give a light red or yellowish-brown solution, which contains the cyan-compound of haematin. The spectrum of haematin in alkaline solution will appear as a broad, faint band.

An ammoniacal solution of carmine gives two absorption-bands similar to those of oxyhaemoglobin, but they change upon addition of acetic acid or ammonium sulphide. Fuchsine gives a band analogous to that of haemoglobin but it remains unchanged when shaken with air.

Cold water dissolves methaemoglobin from freshly dried blood stains. It may be detected by a spectroscopic examination.[1]

OTHER TESTS FOR BLOOD.

The following additional tests may be employed for the detection of blood:

1. **Schönbein-Van Deen Ozone Test.**—Ozonized turpentine[2] and an alcoholic tincture of guaiac resin, mixed and

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[1] See "Handbuch der physiologisch- und pathologisch-chemischen Analyse für Aerzte und Studirende von Felix Hoppe-Seyler," 6th edition, page 412.

[2] Turpentine always contains ozone, when it has been exposed to light for a long time in a bottle which is not tightly stoppered.

shaken with a small quantity of blood, will produce a light blue color. The tincture will separate from the turpentine with a deep blue color. This test is exceedingly delicate, but not characteristic of blood, since many inorganic and organic substances, under the same conditions, produce "guaiac-blue." Free nitrous acid, free chlorine, bromine and iodine, chromic and permanganic acids, ferric and cupric salts produce blue solutions with guaiac resin direct. In examining stains for blood, these substances can usually be excluded beforehand. But other substances, which, like cell-contents or haemoglobin, have the power of transferring ozone, may give a false significance to the guaiac-blue reaction. The enzymes (diastases), hydrolytic ferments (enzymes in the narrower sense) and also the so-called oxidation-ferments (oxidases) are organic substances which may be mentioned. They are found in many vegetable materials, especially in fungi and in the seeds of plants. Saliva, extracts of certain organs, contents of white blood-corpuscles and pus-cells are animal materials which behave similarly. E. Schaer[1] states that these animal and vegetable substances, unlike hydrogen dioxide, are both catalytic in action and carriers of oxygen. And further, that a temperature of 100°, or contact with hydrocyanic acid, completely destroys their power of transferring oxygen, or at least greatly diminishes it, in which respect they essentially differ from haemoglobin! Neither high temperature (100°), nor hydrocyanic acid, has any restraining influence upon haemoglobin in so far as transference of oxygen is concerned. If therefore, an extract containing one of these ferment-like substances, but no blood, be kept even for a short time in a hot water-bath, it will lose the power of giving the "guaiac blue" test. Likewise, the result will be negative, if a test be made in which the stain has been extracted in presence of hydrocyanic acid. Consequently, the greatest care must be observed in drawing conclusions from a positive guaiac test, obtained with the extract of a stain. The guaiac test is certainly very useful as a delicate preliminary test, and in many instances as a check upon blood. The three forms of the blood-pigment which enter into such an examination, namely, haemoglobin, methaemoglobin and haematin are alike, at least qualitatively, in the guaiac test as far as transference of oxygen is concerned. Hence, the analysis and extraction of the stain may be conducted in neutral, acid or alkaline solution, depending upon the nature of the substance, and either hot or cold. An alkaline extract must be rendered faintly acid with acetic acid before guaiac tincture is added. Certain cases seem to demand the extraction of the blood-stain with acidified alcohol. Such an acid, alcoholic haematin solution may be mixed directly with guaiac tincture. Addition of water will precipitate the resin with the blood-pigment adhering to it.

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[1] *Forschungsberichte über Nahrungsmittel,* etc., 3, 1 (1896) and *Archiv der Pharmacie,* 236, 571 (1898).

(*a*) **Vitali's Procedure.**—Extract the stain with water containing carbon dioxide or, in the case of old stains, with very dilute sodium hydroxide[1] solution which is free from nitrite and nitrate. Filter the extract, if necessary, and, after acidifying with acetic acid, add a small quantity of alcoholic guaiac tincture to a portion of the filtrate. If the milky liquid show no blue color in 15 minutes, it contains no oxidizing agents which can interfere with the result. Then add a few drops of old turpentine and shake. If blood-pigment be present, the milky liquid will turn blue either immediately or in a short time. Very gentle warming upon the water-bath increases the delicacy of the reaction. Even putrid blood two months old should give a positive test.

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[1] Sodium hydroxide, prepared from metallic sodium, should be used in this test.

(*b*) **E. Schaer's Procedure.**—Chloral hydrate solution (70 per cent.), allowed to stand for some time in contact with blood-stains upon linen, even when they are very old, will dissolve them completely. Moistening the stains beforehand with glacial acetic acid will facilitate solution. At the same time, prepare an extract of guaiac resin in chloral hydrate solution (70 per cent.). Mix equal volumes of this solution and the extract of the stain. In the absence of nitrates, this mixture will have a brownish-yellow to light brown color. It may be used in making a zone-test for blood. Add Hünefeld's[1] turpentine solution, or hydrogen peroxide, as a surface-layer upon this mixture of blood and guaiac. An intense, blue zone will appear where the two solutions meet. Guaiaconic acid in the guaiac resin produces " guaiac-blue." O. Döbner has suggested substituting a dilute solution of guaiaconic acid for guaiac resin. Blood, or blood-pigment, behaves like a ferment and renders active the " ozonized turpentine," or hydrogen peroxide, either of which by itself will not turn the solution of guaiac resin blue.

2. **E. Schaer's Aloïn Test for Blood.**—The same conditions, which produce " guaiac-blue " from guaiaconic acid, will form " aloïn-red " from aloïn. This substance is characterized by its strong coloring power, and is superior to " guaiac blue " because it lasts longer. The same solution of blood in 70 to 75 per cent. chloral hydrate solution, mixed with a weak chloral solution of aloïn, may be used. Pour a layer of Hünefeld's solution upon the surface or, if it be desirable to produce the color more quickly and more beautifully, use hydrogen peroxide. After some time, a violet-red zone will appear, and a red color of equal intensity will gradually extend throughout the aloïn solution. Another method of performing this test consists in first extracting

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[1] Directions for preparing this reagent are given upon page 216.

the blood-stain with pure water, acetic acid, chloral hydrate solution or alkaline salt solution. After neutralization, add dilute, alcoholic aloïn solution and hydrogen peroxide to this solution. A red color will appear immediately and persist for a long time.

3. Biological Detection of Human Blood.[1]

Specific, bacteriolytic substances can be produced by the injection of bacteria, and in the same way specific, haemolytic and agglutinating bodies can be obtained by injecting the blood of one animal species into an animal of a different species. If rabbit's blood, for instance, be injected into a guinea-pig in repeated doses, the serum of such a guinea-pig will contain substances, which will agglutinate and dissolve the red corpuscles of the rabbit, setting haemoglobin free and rendering the blood laky. The blood-serum of an animal, into which defibrinated blood, or blood-serum from a different animal species, has been injected intravenously, subcutaneously or intraperitoneally, that is to say, into the peritoneal cavity, has the peculiar property of causing precipitation only in the blood-serum of this particular animal species. Uhlenhuth,[2] Wassermann and Schütze,[3] and others, independently of each other, have made experiments of this kind with blood-serum. The object of these experiments has been to introduce into forensic investigations a test, based upon this biological method, for differentiating human blood from the blood of every other animal. Repeated injection of 10 c.c. of defibrinated human blood, or human blood-serum free from cells, into a rabbit, either intraperitoneally or subcutaneously, yields a serum which will produce a heavy, cloudy precipitate in an aqueous solution of human blood. The action of this precipitin is specific. It will produce a precipitate, only in presence of human blood! Wassermann and Schütze tested the blood of 23 different animals, among which were mammals, birds and fishes, but they obtained negative results with blood solutions from these very different animal species. Even old human blood, which has been dried for many weeks, can be differentiated from the blood of other animals by means of blood-serum.

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[1] This subject has been introduced for the sake of completeness. If such an investigation be for legal-chemical purposes, the chemist either will decline to undertake it, or will conduct the experiment with an associate who has had bacteriological and pathological experience.

[2] *Deutsche medizinische Wochenschrift,* 1901, No. 6, and *Zeitschrift für Medizinalbeamte,* 1903, Heft 5 and 6.

[3] *Berliner klinische Wochenschrift,* 1901, No. 7.

A. Dieudonné[1] employs 1 per cent. blood solutions in demonstrating the use of this method. He places 2 c.c. of the clear, filtered solution in small test-tubes, and adds the same quantity of double, physiological salt-solution (= 1.6 per cent. NaCl). Six drops of serum are added to these solutions, and the tubes are placed in the incubator at 37°. The serum of the rabbit, which had been treated with human blood-serum, added to an aqueous solution of human blood, produced in a few minutes a distinct, flocculent precipitate, which gradually became more and more marked. An experiment should be made with normal rabbit's serum as a check. This will produce no precipitate in a solution of human blood. Dieudonné has also found that rabbit's serum, obtained after injecting human blood-serum, will cause precipitation, not only with a solution of human blood but with human urine containing albumin, with an exudate from human pleura and with peritoneal exudate. But precipitation in the case of human blood was much more marked than in these other tests. In his experiments Dieudonné used blood, expressed from the placenta, which was repeatedly injected subcutaneously into rabbits in separate doses of 10 c.c., and at intervals of 3 or 4 days. The animals were bled several days after the last injection, and the blood was kept upon ice.

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[1] *Münchener medizinische Wochenschrift*, 1901, page 533.

The antiserum, to be used in detecting blood, must be absolutely clear, above everything else. Such serum may be obtained by using a sterile Berkefeld filter, which can be connected with a pump attached to any water-pipe. The antiserum must be active in very dilute solution. A distinct turbidity must appear immediately in a solution diluted 1 : 1000, or at latest in 1 or 2 minutes. Sera must be thus highly efficient, to be of practical use. Uhlenhuth has shown that the biological method for detecting blood is a general specific for human albumin. Consequently, blood must be recognized as such, first of all. The first question for the expert to answer in such an investigation always is: "Is there any blood at all present?" If the answer be affirmative, the next question is: "Is it human or animal blood?" Accordingly, the material first of all must be examined for blood-stains by van Deen's ozone test, Teichmann's haemin test and by the spectroscope. If the suspected stains be upon a hard surface, such as a knife, hatchet, gun-barrel, wood, stone, etc., they must be scraped off for the biological blood test, and extracted for several hours in a test-tube with physiological salt solution (= 0.8 per cent. NaCl). First, filter the extract through paper. Should the solution not be clear, pass it through a Berkefeld filter.

CHAPTER V.

QUANTITATIVE ESTIMATION OF ALKALOIDS AND OTHER DRUGS IN CRUDE MATERIALS.

ESTIMATION OF CANTHARIDIN IN SPANISH FLIES.

Pour 100 grams of chloroform and 2 c.c. of hydrochloric acid upon 25 grams of Spanish flies, which have been ground mediumly fine and placed in an Erlenmeyer flask. Shake the mixture frequently during 24 hours. Then pour 52 grams of the chloroform solution through a dry filter, kept well covered, and collect the filtrate in a weighed flask holding 80 to 100 c.c. Distil the chloroform, and add 5 c.c. of petroleum ether to the residue. Stopper the flask, and let the mixture stand 15 hours, with occasional agitation. Dry at 100° and weigh a filter (5 cm. in diameter). Pass the liquid through this filter, having first moistened it with petroleum ether. Treat the undissolved residue twice with petroleum ether, each time using 10 c.c. and shaking. Pass this solvent through the same filter, and disregard crystals adhering to the side of the flask. Dry the filter and flask, and wash both with a small quantity of water, containing a drop of ammonium carbonate solution to every 10 c.c., until this solvent has only a faint yellow color. Finally, wash once with 5 c.c. of water, and dry both flask and filter. Place the filter and contents in the flask, and dry at 100° to constant weight. The crystalline residue should weigh at least 0.1 grams.

Cantharidin ($C_{10}H_{12}O_4$) appears in tabular crystals which melt at 218°. It is almost insoluble in water; slightly soluble in alcohol, ether and benzene; and quite freely soluble in chloroform and acetone. When heated for some time with alkalies, cantharidin adds a molecule of water and forms cantharidic acid, or rather the alkali salts of this acid. Addition of acids to salts of cantharidic acid causes separation of cantharidin. Hence, cantharidin is considered to be the anhydride of cantharidic acid. Santonin and santonic acid are similarly related. Some cantharidin is present in Spanish flies as a salt of cantharidic acid. To include this portion also in the estimation, the Spanish flies must be moistened with hydrochloric acid, and allowed to stand for several hours. There is no chemical test characteristic of cantharidin. It must be identified by its physiological action. It has a very irritating action and will blister the skin. In making this test, dissolve some of the material, preferably in hot olive oil. Apply a piece of linen moistened with this solution to the upper part of the arm or chest, and hold it in place with adhesive plaster. If cantharidin be present. it will soon redden and blister the skin under the linen.

Estimation of Alkaloid in Cinchona Bark.

1. **Keller-Haubensack Method.**—Mix 12 grams of finely powdered, dry cinchona bark in an Erlenmeyer flask with 120 grams of ether. Then add 10 grams of ammonium hydroxide solution (10 per cent. NH_3), and shake thoroughly at frequent intervals during 30 minutes. Add water (10 grams for red cinchona bark and 15 grams for calisaya bark), and again shake vigorously for a minute. Pour 100 grams of the clear ether solution into another Erlenmeyer flask, add 3 c.c. of dilute sulphuric acid and 37 c.c. of water, shake vigorously for several minutes and let the mixture settle. Remove most of the ether, and pour the remainder of the mixture into a separating funnel. Withdraw the acid, aqueous solution of the alkaloidal sulphates, and wash flask and separating funnel with 10 c.c. of water. Return the

acid, aqueous solution to the separating funnel, and add 6 grams of ammonium hydroxide solution. Extract with a mixture of 10 grams of ether and 30 grams of chloroform, and after removing the ether-chloroform layer, repeat the extraction with 5 grams of ether and 15 grams of chloroform. To remove water, pour the combined ether-chloroform extracts through a dry filter into a dry, weighed flask. Wash the filter with the ether-chloroform mixture and distil. Calisaya bark usually gives crystalline alkaloids at once, whereas red cinchona bark yields a varnish which persistently retains chloroform. In the latter case, pour 3 to 5 c.c. of absolute alcohol over the residue, and evaporate upon the water-bath. The alkaloids will become crystalline, and then they can be easily dried. Finally dry to constant weight.

2. **Method Directed by the German Pharmacopoeia.**—To determine total alkaloids, pour 90 grams of ether and 30 grams of chloroform upon 12 grams of finely ground cinchona bark, dried at 100° and placed in an Erlenmeyer flask. Add 10 c.c. of sodium hydroxide solution. Shake vigorously at frequent intervals during 3 hours. Then add 10 c.c. of water, or enough to cause the powdered cinchona to gather into lumps after vigorous shaking, thus leaving the supernatant ether-chloroform solution perfectly clear. Let the ether-chloroform solution stand an hour, and then pass 100 grams through a dry filter, kept well covered. Collect the filtrate in a flask, and distil half the solvent. Pour the remaining ether-chloroform solution into a separating funnel, and wash the flask three times with 5 c.c. portions of a mixture of 3 parts of ether and 1 part of chloroform. Thoroughly extract the total ether-chloroform solution with 25 c.c. of N/10 hydrochloric acid. When the contents of the separating funnel are perfectly clear, add enough ether to bring the ether-chloroform solution to the surface. Pass the acid solution through a small filter moistened with water, and receive the filtrate in a 100 c.c. flask. Make three more extractions of the ether-chloroform solution with 10 c.c. portions of water, and pass these extracts through the same filter. Wash the filter with water, and bring the volume of the filtrate to 100 c.c. Finally, measure 50 c.c. of this solution with a pipette, and add freshly prepared haematoxylin solution, made by dissolving a small particle of this substance in 1 c.c. of alcohol. Shake and add enough N/10 potassium

hydroxide solution to give the mixture a yellowish color, which quickly changes after vigorous agitation to bluish-violet. This should require not more than 4.3 c.c. of the alkaline solution.

Aqueous Cinchona Extract (I) and Alcoholic Cinchona Extract (II).—To determine total alkaloids in these preparations, dissolve 2 grams of (I) or (II) in an Erlenmeyer flask, using 5 grams of water and 5 grams of absolute alcohol. Add 50 grams of ether and 20 grams of chloroform, and, after vigorous shaking, 10 c.c. of sodium carbonate solution (1 : 3). Shake frequently, and let the mixture stand an hour. Then pass 50 grams of the ether-chloroform solution through a dry filter, kept well covered. Receive the filtrate in a flask, and distil half the solvent. Pour the remainder into a separating funnel, wash the flask three times with 5 c.c. portions of a mixture of 3 parts of ether and 1 part of chloroform, and then shake the total ether-chloroform solution with 10 c.c. of N/10 hydrochloric acid. When the contents of the separating funnel are perfectly clear, add enough ether to bring the ether-chloroform solution to the surface. Pass the acid solution through a small filter moistened with water, and receive the filtrate in a 100 c.c. flask. Make three more extractions of the ether-chloroform solution with 10 c.c. portions of water, and pass these extracts through the same filter. Wash the filter with water, and bring the volume of the filtrate to 100 c.c. Finally, measure 50 c.c. of this solution with a pipette, and add freshly prepared haematoxylin solution, made by dissolving a small particle of this substance in 1 c.c. of alcohol. Shake and add enough N/10 potassium hydroxide solution to give the mixture a yellowish color, which quickly changes upon vigorous agitation to bluish-violet. This should require not more than 3.7 c.c. for (I), nor more than 2.3 c.c. for (II).

Estimation of Caffeïne in Coffee, Tea, Cola Nuts, Etc.

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[1] From 1897 to 1902 (inclusive).

[2] C = *Chemisches Centralblatt.*

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K. Dieterich.—Ueber die Werthbestimmung der Kolanuss und des Kolaextraktes. Vortrag auf der Naturforscherversammlung in Braunschweig gehalten. Pharmaceutische Zeitung 42, 647–650 and C 1897 II, 977.

H. Brunner and H. Leins.—Ueber die Trennung und quantitative Bestimmung des Kaffeïns und Theobromins. Schweizer Wochenschrift für Pharmacie 36, 301–303 and C 1898 II, 512.

J. Gadamer.—Ueber Kaffeïnbestimmungen in Tee, Kaffee und Kola. Archiv der Pharmacie 237, 58–68 and C 1899 I, 713.

F. Katz.—Ueber die quantitative Bestimmung des Kaffeïns. Berichte der Deutschen pharmaceutischen Gesellschaft 12, 250 (1902).

1. C. C. Keller's Method.—Pour 120 grams of chloroform upon 6 grams of dry, unbroken tea leaves[1] in a wide-mouthed separating funnel. In a few minutes, add 6 c.c. of ammonium hydroxide solution (10 per cent. H_3N), and at frequent intervals shake vigorously during 30 minutes. Then let the separating funnel stand at rest, until the solution is perfectly clear and the tea-leaves have absorbed all the water. This may require 3 to 6 hours, or even longer depending upon the variety of tea. Pass 100 grams of clear chloroform extract, representing 5 grams of tea, through a small filter moistened with chloroform. Receive the filtrate in a small, weighed flask and distil the chloroform upon the water-bath. Pour 3 or 4 c.c. of absolute alcohol over the residue. Heat upon the water-bath to remove alcohol and expel alcohol vapor with a hand-bellows. In a few minutes the caffeïne will be dry and at the same time free from imprisoned chloroform. In a measure also, this treatment with alcohol separates caffeïne from extraneous chlorophyll. The latter adheres to the bottom and side of the flask, whereas caffeïne forms a white incrustation upon it. Caffeïne thus obtained is usually impure from small quantities of ethereal oil, fat, vegetable-wax and principally chlorophyll. Consequently, it must be purified. Set the flask upon a boiling water-bath and pour a mixture of 7 c.c. of water and 3 c.c. of alcohol over the crude caffeïne, which upon being shaken will pass into solution almost immediately. Then add 20 c.c. of water, stopper the flask and shake vigorously. The chlorophyll will form lumps and the solution will filter easily. Pass the caffeïne solution through a small filter moistened with water, wash flask and filter with 10 c.c. of water, evaporate the total filtrate in a weighed glass dish to dryness upon the water-bath and weigh the residue of nearly pure caffeïne. The weight of this residue multiplied by 20 will give the percentage of caffeïne in the tea.

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[1] When a wide-mouthed separating funnel cannot be obtained, triturate the tea-leaves somewhat, solely to facilitate their removal from the separating funnel after extraction. Finely powdered tea is not only unnecessary, but even objectionable, because the extracts have a much deeper color, and the yield of caffeïne is not increased.

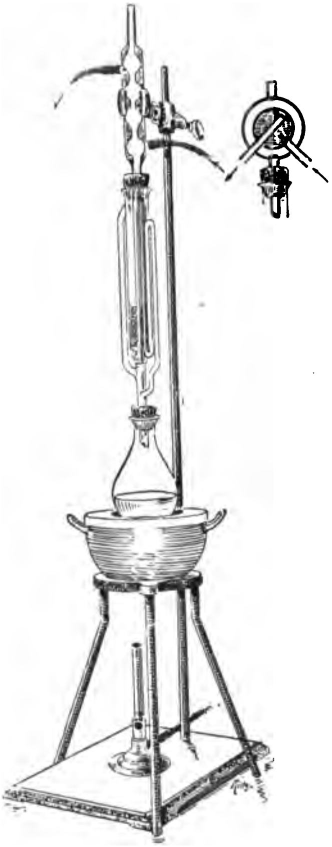
Notes.—Ammonia causes tea-leaves to swell considerably, and at the same time it combines with the tannic acid present. Caffeïne is set free and dissolved by chloroform. The color of the chloroform extract depends upon the variety of tea. Black teas (Pekoe, Souchong and Congo) give clear, pale-green to yellowish-green solutions. Teas not so black, or green teas, give darker and more brownish-green solutions. In assaying those varieties of tea, which probably contain a small quantity of caffeïne, take 12 grams and extract with 150 grams of chloroform. C. C. Keller has shown that the best and most expensive varieties of tea contain the most caffeïne. The average percentage of caffeïne, based upon 50 assays of tea, was found to be 3.06. A green tea gave the smallest yield, namely, 1.78 per cent. of caffeïne; and a Pekoe tea the highest yield, namely, 4.24 per cent. of caffeïne.

J. Gadamer states that Keller's method of estimating caffeïne in tea is applicable also to coffee and cola preparations. Keller's method is especially useful for roasted coffee. The caffeïne, though somewhat brown, is always sufficiently pure.

2. **Hilger-Juckenack Method.**—Macerate 20 grams of finely ground coffee, or triturated tea, with 900 grams of water for several hours in a large beaker, at room temperatures. Then boil thoroughly and replace the water lost by evaporation. Raw coffee will require 3 hours and roasted coffee and tea 1.5 hours. Cool somewhat (60 to 80°) and add 75 grams of aluminium acetate solution (see note, page 200) and gradually, while stirring, 1.9 grams of acid sodium carbonate. Boil about 5 minutes and bring the total weight

when cold to 1020 grams. Filter 750 grams (= 15 grams of original material) and add to the clear filtrate 10 grams of precipitated and powdered aluminium hydroxide and some filter-paper made into a magma by agitation with water. Stir frequently and evaporate upon the water-bath. Thoroughly dry the residue in an air-closet at 100°, and extract for 8 to 10 hours with pure tetrachloromethane (CCl_4) using a Soxhlet apparatus (Fig. 17). Tetrachloromethane, which is always colorless, is finally distilled and the residue of perfectly white caffeïne is dried at 100° and weighed. The results thus obtained are usually accepted without question. But, if an absolutely accurate result be required, nitrogen in the crude caffeïne may be determined by Kjeldahl's method. The quantity of anhydrous caffeïne is calculated on the basis of this analysis. One c.c. of N/10 oxalic acid represents 0.00485 grams of anhydrous caffeïne. Commercial tetrachloromethane is usually impure and cannot be used directly in the extraction. It should be shaken 3 or 4 times with sodium carbonate solution, then several times with water, dried over fused calcium chloride and distilled fractionally. It boils at 76 to 77°.

FIG. 17.



Soxhlet Apparatus.

3. Trillich-Goeckel Modification of Hilger's Method. —Exhaust 10 grams of finely ground coffee with water. This will require 3 extractions with boiling water, using 200 c.c. portions and heating 30 minutes. Combine the filtered extracts, cool and dilute to 495 c.c. Add 5 c.c. of basic lead acetate solution, shake thoroughly, filter 400 c.c. and pass hydrogen sulphide through the filtrate. Dilute this filtrate to 500 c.c., shake thoroughly and again filter 400 c.c. This filtrate will represent 6.4 grams of coffee. Concentrate this filtrate upon the water-bath, and, after addition of 1 gram of magnesium oxide and sand, evaporate to dryness. Triturate the residue and extract for 30 hours with acetic ether, using a Soxhlet apparatus. Evaporate the acetic ether extract in a Kjeldahl flask, or distil the solvent, and determine nitrogen in the residue by Kjeldahl's method. One gram of nitrogen represents 3.4643 grams of caffeïne. Crude caffeïne is easily decomposed by the acid used in the Kjeldahl process and by mercuric oxide. Roasted coffee may easily give too much caffeïne by this method, because the bases formed by roasting coffee, pyridine for example, are also extracted by acetic ether.

4. Trillich-Goeckel Modification of Socolof's Method. —Put 10 grams of finely ground, dried coffee into a separating funnel, provided with a plug of glass wool for a filter, and moisten with ammonium hydroxide solution. Let the mixture stand for 30 minutes, extract for 12 hours with 200 c.c. of acetic ether and shake frequently. Filter, and wash three times with 50 c.c. portions of acetic ether. Distil the acetic ether upon the water-bath and boil the residue with milk of magnesia. Filter, and evaporate the filtrate to dryness upon the water-bath. Dissolve the residual caffeïne in acetic ether or chloroform. Filter this solution into a weighed dish or Kjeldahl flask. Evaporate the solvent and weigh the caffeïne, or calculate it from the percentage of nitrogen. The latter method is the more accurate. Determination of nitrogen by Kjeldahl's method is the surest method of estimating caffeïne in the extract.

5. E. Katz's Method of Estimating Caffeïne.—This method is based upon the fact that chloroform will extract caffeïne quantitatively from a solution which is ammoniacal, or faintly acid with hydrochloric acid.

Shake 10 grams of powdered coffee, or tea, for 30 minutes with 200 grams of chloroform and 5 grams of ammonium hydroxide solution. When the liquid has settled, filter 150 grams of the chloroform solution which should be perfectly clear and free from water. Distil the chloroform and dissolve the residue with gentle heat in about 6 c.c. of ether. Add 20 c.c. of 0.5 per cent. hydrochloric acid and, in an assay of coffee, also 0.2 to 0.5 grams of solid paraffin. Evaporate the ether and filter the cold, aqueous solution. Wash the flask and filter-paper a few times with small portions of 0.5 per cent. hydrochloric acid. Finally extract the total aqueous hydrochloric acid solution four times with 20 c.c. portions of chloroform. Distil the filtered chloroform extract, dry the residue and weigh. This residue will consist of nearly pure caffeïne. J. Katz found the following percentages of caffeïne:

	Caffeïne.	Average.
Raw Coffee Beans:	0.9 –1.27 per cent.	1.14 per cent.
Dried Cola Nuts:	1.51–1.94 "	1.68 "
Black Tea:	2.51–3.56 "	3.07 "
Guarana Paste:	2.83–4.74 "	4.08 "

J. Katz recommends the following method for estimating caffeïne in maté or Paraguay tea:

Treat finely triturated tea with ammonium hydroxide solution and chloroform, as described above, and dissolve the chloroform residue in ether. Add water to the ether and evaporate. Warm the aqueous solution 10 minutes upon the water-bath with 2 c.c. of lead hydroxide suspended in water (1:20). If it be very difficult to get a clear filtrate from this liquid, add a small quantity of calcined magnesium oxide. This treatment usually gives a filtrate, which is perfectly clear when cold, and but slightly colored. Chloroform will extract quite pure caffeïne from this solution. By this method maté yields 0.3 to 1.6 per cent. of caffeïne, the average being 0.71 per cent.

6. K. Dieterich's Method of Estimating Total Alkaloids (Caffeïne and Theobromine) in Cola-Nuts.—Moisten 10 grams of finely grated Cola-nuts with a small quantity of water, mix with 10 grams of granulated, unslaked lime and extract with chloroform in a Soxhlet apparatus for 45 minutes. Evaporate the extract almost to dryness and dissolve the residue with gentle heat in 20 c.c. of N hydrochloric acid. Filter and dilute to 100 c.c. Add ammonium hydroxide solution in large excess to this filtrate, shake at frequent intervals during 15 minutes and extract three times with 20 c.c. portions of chloroform. Evaporate this chloroform solution and dry the residue, which usually consists of perfectly pure caffeïne, to constant weight.

This method may also be used in estimating caffeïne in Paraguay tea. Mix the finely ground material with unslaked lime, and extract with chloroform in a Soxhlet apparatus.

ESTIMATION OF ALKALOIDS IN IPECAC (RADIX IPECACUANHAE).

Ipecac has been shown[1] to contain three alkaloids, namely, cephaëline ($C_{28}H_{40}N_2O_4$), emetine ($C_{30}H_{44}N_2O_4$) and psychotrine. The composition of the last alkaloid is unknown. This drug acts as an expectorant and emetic, because of cephaëline and emetine. Psychotrine is said not to possess these properties. Therefore, in assaying ipecac for medicinal purposes, only the percentage of the first two alkaloids need be estimated. The equivalent weights of these two alkaloids (cephaëline 234 and emetine 248) are so nearly the same, that the mean of the two (241) may be used as the factor.

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[1] Frerichs and de Fuentas Tapis, *Archiv der Pharmacie,* 1902, Heft 5 and 6.

Procedure.—Put 6 grams of finely powdered root in a dry Erlenmeyer flask and shake with 60 grams of ether. Then add 5 c.c. of ammonium hydroxide solution, or 5 c.c. of sodium carbonate solution (1 : 3), and shake frequently during an hour. Add 10 c.c. of water and, after shaking vigorously, filter 50 grams of ether into a small flask. Evaporate half the ether upon the water-bath, and extract the remainder in a separating funnel with 10 c.c. of N/10 hydrochloric acid. Pass the acid solution through a small filter into a 200 c.c. flask. Make two more extractions of the ether with 10 c.c. portions of water, and pass these through the same filter. Bring the volume of the acid solution to 100 c.c., and then add enough ether to form a layer about 1 cm. thick after thorough agitation. Add 5 drops of iodeosine solution (1:250), and titrate excess of hydrochloric acid with N/10 potassium hydroxide solution. The number of c.c. of N/10 hydrochloric acid combined with the alkaloids, multiplied by 0.0241, will give the quantity of emetine and cephaëline in 5 grams of ipecac.

To estimate these alkaloids gravimetrically, shake vigorously the ether solution of the alkaloids (50 grams = 5 grams of root) in a separating funnel with 5 c.c. of dilute hydrochloric acid and 10 c.c. of water. Transfer the acid solution to another separating funnel. Make two more extractions of the ether with 10 c.c. portions of water and add these to the acid extract. Add 5 c.c. of ammonium hydroxide solution to the acid extract and shake vigorously with 50 grams of ether. Remove the aqueous layer and filter 40 grams of the ether solution into a weighed flask. Evaporate the ether and weigh the flask after drying for an hour at 100°. This will give the quantity of emetine and cephaëline in 4 grams of root.

Test for Cephaëline.—This reaction is very characteristic of this alkaloid. Froehde's reagent dissolves pure cephaëline, as the free base, almost without color. Add a trace of hydrochloric acid, or better sodium chloride, to this solution and it will produce an intense blue color. Pure emetine gives no color with Froehde's reagent, nor when sodium chloride is added. This test for cephaëline may be made with the ether residue.

The method of estimating alkaloids in ipecac, as directed in the "German Pharmacopoeia," is the same as that for cinchona bark. Use 12 grams of finely powdered root dried at 100°, but in ascertaining the excess of acid use iodeosine, and not haematoxylin, as the indicator. Finally, measure with a pipette 50 c.c. of the proper solution having a volume of 100 c.c., place in a 200 c.c. flask and add about 50 c.c. of water and enough ether to make a layer 1 cm. thick. Add 5 drops of iodeosine solution and enough N/100 potassium hydroxide solution, shaking thoroughly after each addition, to give the lower aqueous layer a pale red color. This should require not more than 20 c.c. of alkaline solution.

ESTIMATION OF NICOTINE IN TOBACCO.

1. **R. Kissling's[1] Method.**—First remove the ribs and then cut the tobacco-leaves into small pieces. Dry 1 or 2 hours (50 to 60°), and then reduce to a uniform, coarse powder. Triturate 20 grams of this powder with 10 c.c. of dilute, alcoholic sodium hydroxide solution (6 grams of sodium hydroxide dissolved in 40 c.c. of water and 60 c.c.

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[1] *Zeitschrift für analytische Chemie,* 34, 1731 and 21, 76.

of 95 per cent. alcohol). Transfer this moist powder to a paper thimble and extract with ether in a Soxhlet apparatus. Carefully distil the ether solution so that a portion of the solvent remains. Add very dilute sodium hydroxide solution (4 grams of sodium hydroxide in 1000 c.c. of water) to the residue and distil with steam. Begin introducing steam after the nicotine solution has been boiling several minutes. Collect about 400 c.c. of distillate, that is to say, continue distilling until the distillate is no longer alkaline. Mix well, add a few drops of rosolic acid solution to the distillate, and titrate nicotine with N/10 sulphuric or oxalic acid. Each c.c. of N/10 acid represents 0.0162 grams of nicotine.

2. **C. C. Keller's[1] Method.**—Pour 60 grams of ether and 60 grams of petroleum ether over 6 grams of dry tobacco in a 200 c.c. Erlenmeyer flask. Add 10 c.c. of 20 per cent. aqueous potassium hydroxide solution and let the mixture stand half an hour, shaking vigorously at frequent intervals. After the liquid has stood at rest 3 or 4 hours, pour 100 grams of ether solution through a small, plaited filter and receive the filtrate in a 200 c.c. Erlenmeyer flask. Nicotine will be in solution together with a small quantity of ammonia, which must be removed before titration. By means of a hand bellows and a glass tube reaching to the bottom of the flask force a current of air through the solution, so that there is considerable agitation. It will require about a minute and a half to expel all ammonia. At the same time, 8 to 10 grams of ether will evaporate. Add 10 c.c. of alcohol, a drop of 1 per cent. iodeosine solution and 10 c.c. of water to the ammonia-free solution. Stopper the flask and shake vigorously. Nicotine and iodeosine will dissolve in the water which will have a red color. Add a

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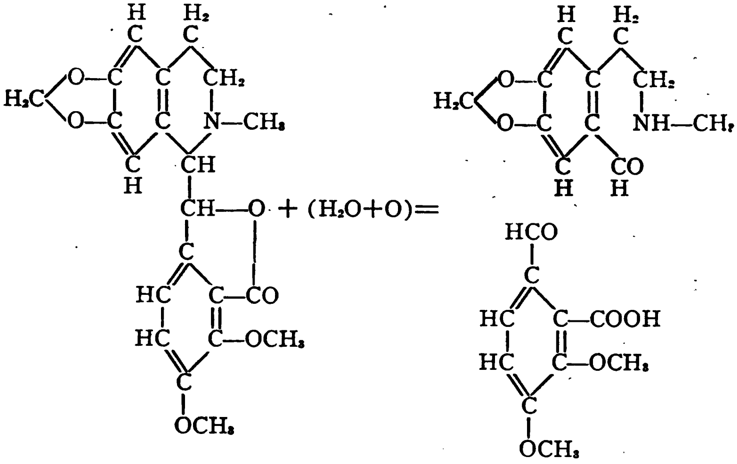
[1] *Berichte der Deutschen pharmaceutischen Gesellschaft,* 8, 145 (1898).

slight excess of N/10 hydrochloric acid, enough to discharge the color, and titrate excess of acid with N/10 potassium hydroxide solution. The quantity of nicotine in tobacco shows a wide variation and ranges from 0.6 to 4.8 per cent.

ESTIMATION OF HYDRASTINE IN FLUID EXTRACT OF HYDRASTIS.

Evaporate 15 grams of fluid extract of hydrastis to about 5 grams in a weighed dish upon the water-bath, and wash the residue into an Erlenmeyer flask with about 10 c.c. of water. Add 10 grams of petroleum ether, 50 grams of ether and 5 grams of ammonium hydroxide solution. Let the mixture stand an hour, shaking vigorously at frequent intervals. Then pass 50 grams of the clear ether solution through a dry filter into a separating funnel. Add 10 c.c. of a mixture, composed of 1 part of hydrochloric acid and 4 parts of water, and shake the solution vigorously several minutes. When the liquids have separated clear, run the acid solution into an Erlenmeyer flask. Make two more extractions of the ether with 5 c.c. portions of water containing a few drops of hydrochloric acid, and add these to the first extract. Add to the total extract excess of ammonium hydroxide solution and 50 grams of ether. Let the mixture stand an hour, shaking vigorously at frequent intervals. Pass 40 grams of the clear ether solution through a dry filter and collect the filtrate in a weighed, dry flask. Distil the ether, dry the residue at 100° and weigh when cold. The residue should weigh at least 0.2 grams. Dissolve the residue in 10 c.c. of water, at the same time adding a few drops of dilute sulphuric acid. Add 5 c.c. of potassium permanganate solution and shake as long as there is color. This will give a blue, fluorescent solution.

Hydrastine ($C_{21}H_{21}NO_6$) crystallizes from alcohol in prisms and melts at 132°. It is insoluble in water, somewhat soluble in alcohol and freely soluble in benzene and chloroform. It is a tertiary base, has an alkaline reaction and is not very poisonous. Its constitution has been determined. Hydrastine is very closely related to narcotine, which very probably is a methoxy-hydrastine. Hydrastine has two methoxyl-groups as shown by Zeisel's[1] method. The oxidation products of hydrastine are nearly the same as those of narcotine. Chromic acid, or manganese dioxide and sulphuric acid, decompose hydrastine into opianic acid and the base hydrastinine ($C_{11}H_{13}NO_3$).



Hydrastinine.

Hydrastine. Opianic Acid.

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[1] It is a remarkable fact that by far the greater number of alkaloids contain one or more, sometimes three or more, methoxyl-groups (—$O.CH_3$), united with a benzene nucleus, and the determination of the number of such groups in the molecule is of the greatest importance as a step in establishing the constitution of an alkaloid, because in this way some of the carbon, oxygen and hydrogen atoms are at once disposed of. The method employed for this purpose depends on the fact that all substances containing methoxyl-groups are decomposed by hydriodic acid, yielding methyl iodide and a hydroxyl-compound. By estimating the methyl iodide obtained from a given quan-

Hydrastinine ($C_{11}H_{13}NO_3$) crystallizes in needles which melt at 116 to 117°. It is a mon-acid, secondary base without a methoxyl-group, but having a methyl-group attached to nitrogen. It has styptic properties. Hydrastinine is slightly soluble in water, but very easily soluble in organic solvents like alcohol, ether and chloroform. As a secondary base, hydrastinine forms an acetyl and benzoyl compound.

ESTIMATION OF MORPHINE IN OPIUM.

(Method of German Pharmacopoeia.)

Triturate 6 grams of rather finely powdered opium with 6 grams of water. Wash the mixture into a weighed, dry flask with water and add enough more of this solvent to bring the weight to 54 grams. Shake frequently and let the mixture stand an hour. Pour upon a piece of dry linen and express the liquid. Pass 42 grams of this extract through a dry, plaited filter (10 cm. in diameter) into a dry flask. Add 2 grams of sodium salicylate solution (1:2) to this filtrate and shake vigorously. Filter 36 grams of the clear solution through a dry, plaited filter (10 cm. in diameter) into a small flask.* Mix this filtrate by gentle agitation with 10 grams of ether, and add also 5 grams of a mixture consisting of 17 grams of ammonium hydroxide solution and 83 grams of water. Stopper the flask, shake vigorously for 10 minutes and let the mixture stand at rest 24 hours. Then decant the ether layer as completely as possible upon a smooth filter. Add 10 grams more of ether to the residual, aqueous liquid in the flask, shake gently and again pour the ether layer upon the filter. Then

tity of a compound of known molecular weight, it is easy, therefore, to determine the number of methoxyl-groups in the molecule. This method was first applied by Zeisel, and is of general application. (Perkin and Kipping, "Organic Chemistry," page 498.) Tr.

pour all of the aqueous solution upon the filter, and disregard crystals adhering to the side of the flask. Wash filter and flask three times with 5 c.c. portions of water saturated with ether. When the filter has drained thoroughly, dry the morphine crystals and dissolve in 25 c.c. of N/10 hydrochloric acid. Pour this solution into a 100 c.c. flask, carefully wash filter and flask with water and finally dilute the solution to 100 c.c. Measure 50 c.c. of this solution into a 200 c.c. flask, add 50 c.c. of water and enough ether to form a layer 1 cm. thick. Add 5 drops of iodeosine solution and enough N/10 potassium hydroxide solution, shaking vigorously after each addition, to produce a pale red color. This should require not more than 5.4 c.c. and not less than 4.1 c.c. of the alkaline solution.

Morphine in Extract of Opium is estimated in the same way. "Dissolve 3 grams of opium extract in 40 grams of water, add 2 grams of sodium salicylate solution (1:2), shake vigorously, and pass 30 grams of clear solution through a dry filter (10 cm. in diameter) and collect in a dry flask." Continue the assay as directed in the preceding method from the point marked with an asterisk. "Not more than 6.5 c.c., nor less than 5.5 c.c., of N/10 potassium hydroxide solution should be required in titration to produce a pale red color."

To determine morphine in Wine of Opium and Tincture of Opium, evaporate about "50 grams in a weighed dish to 15 grams, add water until the weight is 38 grams and also 2 grams of sodium salicylate solution (1:2). Shake vigorously and pass 32 grams of clear solution through a dry, creased filter (10 cm. in diameter) into a dry flask. Mix this filtrate. . . ."* From this point the directions are the same as in the above method. "It should not require more than 5.5 c.c., nor less than 4.2 c.c., of N/10 potassium hydroxide solution to produce the pale red color of the end reaction."

ESTIMATION OF SANTONIN IN WORMSEED.

1. **K. Thaeter's[1] Method.**—Extract 10 grams of crushed wormseed in a Soxhlet apparatus with ether for 12 hours. Distil or evaporate the ether and boil the residue for an hour with 5 grams of lime and 300 c.c. of water. Replace water lost by evaporation. Filter while hot and wash the residue with water. Faintly acidify the filtrate with sulphuric acid and warm until santonin crystals begin to form. Then add 100 grams of aluminium acetate solution,[2] heat the mixture to boiling and finally evaporate to dryness upon the water-bath. Mix the finely powdered residue with 3 grams of magnesium oxide, moisten this mixture with a small quantity of water and again bring quickly to dryness. Powder the residue, dry at 105° and extract in a Soxhlet apparatus with anhydrous and acid-free ether for 5 hours. Santonin will be deposited by evaporating the ether as a faintly yellowish residue which is then weighed.

Remarks.—When wormseed is heated with lime, santonin passes into solution as calcium santonate, and at the same time resinous substances are saponified. Dilute sulphuric acid liberates santonin. Basic aluminium acetate, produced by boiling, precipitates resinous and colored substances. Finally, magnesium oxide serves to neutralize free acetic acid. Under the conditions, practically no magnesium santonate is formed. Thaeter obtained 88 to 92 per cent. of the santonin in the material at the beginning of the assay. Wormseed contains about 2.5 per cent. of santonin.

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[1] *Archiv der Pharmacie*, 237, 626–632 (1899) and 238, 383–387 (1900).

[2] Dissolve 300 parts of aluminium sulphate in 800 parts of water; add acetic acid (sp. gr. 1.041) 360 parts; triturate calcium carbonate 130 parts with 200 parts of water, and add this mixture slowly and with continued stirring to the first solution; set the whole aside for 24 hours without applying heat, and stir occasionally; then strain, press the precipitate without washing it and filter the liquid. It is a clear, colorless liquid, having the sp. gr. 1.044 to 1.046, a faint odor of acetic acid, an acid reaction, and a sweetish, astringent taste. National Dispensatory. Tr.

2. J. Katz's[1] Method.—Extract 10 grams of coarsely powdered wormseed in a Soxhlet apparatus with ether for 2 hours. Distil the ether. There usually remains a dark green resin weighing 1.5 to 2 grams. Boil this residue 15 to 30 minutes, under a reflux condenser, with 5 grams of crystallized barium hydroxide dissolved in 100 c.c. of water. Cool and, without filtering, render the solution acid to litmus with carbon dioxide. Filter immediately with a pump to remove barium carbonate, and wash the precipitate twice with 20 c.c. portions of water. Evaporate the pale yellow solution to about 20 c.c. in a dish upon the water-bath. Add 10 c.c. of 12.5 per cent. hydrochloric acid, continue heating upon the water-bath exactly 2 minutes longer and pour the solution into a separating funnel. Dissolve santonin crystals left in the dish in about 20 c.c. of chloroform. Pour this solution into the separating funnel and shake thoroughly. When the solutions have separated clear, withdraw the chloroform solution and pass it through a dry filter. Wash dish, separating funnel and filter 2 or 3 times with 10 c.c. portions of chloroform. Distil the chloroform and boil the residue 10 minutes, under a reflux condenser, with 50 c.c. of 15 per cent. alcohol. Filter while hot into a weighed flask, and wash flask and filter twice with 15 c.c. portions of 15 per cent. boiling alcohol. Cover the flask and set aside in the cold 24 hours. Weigh flask and contents and pass the latter through a weighed filter, disregarding the milky appearance of the filtrate caused by minute, resinous drops. Wash flask and filter once with 10 c.c. of 15 per cent. alcohol, dry the filter in the flask and weigh both. Finally, apply a correction on account of the solubility of santonin in the alcohol used. Every 10 grams of filtrate contain 6 milligrams of santonin. Santonin by this method is crystalline, and usually of a faint yellow color. J. Katz found the quantity of santonin in wormseed to vary between 1.211 and 3.159 (average 2.42) per cent. This method is based upon the fact that the santonin in 10 grams of wormseed is easily soluble in 50 c.c. of hot 15 per cent. alcohol, whereas only a very small quantity of resin is dissolved by this dilute alcohol. As this dilute, alcoholic solution cools, santonin crystallizes out almost quantitatively.

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[1] *Archiv der Pharmacie,* 237, 251 (1899).

To estimate the quantity of santonin in lozenges, made from this substance and sugar, directly extract the finely ground lozenges with hot chloroform. The santonin can usually be weighed without further purification.

In a somewhat simpler form, the method described above may be used to estimate santonin in chocolate lozenges. Weigh 3 or 4 lozenges and boil 15 minutes under a reflux condenser with 5 grams of barium hydroxide and 100 c.c. of water. Saturate the liquid when cold with carbon dioxide. Filter, wash the residue with water and evaporate the brownish filtrate to 100 c.c. Warm the liquid and add 10 c.c. of dilute hydrochloric acid. Three extractions with chloroform will yield nearly pure santonin. To get santonin crystals almost white and ready for weighing, evaporate the chloroform solution and expel the last traces of chloroform by adding a few c.c. of ether. If santonin be impure from traces of fatty acids, boil once with 10 c.c. of petroleum ether and filter when cold. Santonin is nearly insoluble in cold petroleum ether.

Santonin can be detected and estimated in toxicological analysis in a similar manner. Acidify the material with hydrochloric acid, extract with chloroform and treat the chloroform residue with barium hydroxide solution as described above.

ESTIMATION OF ALKALOIDS IN NUX VOMICA.

(C. C. Keller's[1] Method.)

Remove fat from nux vomica by treating 15 grams of the well-dried and finely powdered drug in a 250 c.c. Erlenmeyer flask three times with 30 c.c. portions of ether. Shake thoroughly for 5 minutes. Pour these ether washings into a flask, and extract dissolved alkaloid with 5 c.c. of N/10 hydrochloric acid and 10 c.c. of water. Repeat the extraction of the ether layer, separated from the aqueous solution, using water instead of acid. Add 100 c.c. of ether, 50 grams of chloroform and 10 grams of 10 per cent. ammonium hydroxide solution to the powdered nux vomica free from fat. Shake thoroughly for 30 minutes and add to this mixture the hydrochloric acid solution used in extracting alkaloid from the first ether washings. Again shake thoroughly and, when the liquids have separated clear, pour 100 grams of ether-chloroform solution through a small filter into a weighed Erlenmeyer flask and weigh. Distil the chloroform and ether as completely as possible. The alkaloids usually appear as colorless varnishes which persistently retain chloroform. To remove the latter, pour a few c.c. of absolute alcohol upon the residue and expel completely upon the water-bath. Repeat this treatment 2 or 3 times. This will give crystalline alkaloids which can be dried at 95 to 100° and weighed.

QUANTITATIVE SEPARATION OF STRYCHNINE AND BRUCINE.

(C. C. Keller's Method.)

Dissolve with gentle heat 0.2 to 0.4 grams of the dry, mixed alkaloids in 10 c.c. of 10 per cent. sulphuric acid.

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[1] Festschrift presented at the fiftieth anniversary of the founding of the Swiss Pharmaceutical Association. Abstract in *Zeitschrift für analytische Chemie*, 23, 491 (1894).

Add 1 c.c. of nitric acid (sp. gr. 1.41 to 1.42) to the cold solution and shake thoroughly. Strychnine sulphate which sometimes crystallizes, as the sulphuric acid solution cools, is usually dissolved again by nitric acid. At the same time, nitric acid produces the red color caused by its action upon brucine. Let the mixture stand 1 to 1.5 hours, to complete the decomposition of brucine. Add 40 grams of chloroform and ether and shake thoroughly. Then add an excess of 10 per cent. ammonium hydroxide solution, and shake vigorously several minutes. Filter 40 grams of ether-chloroform solution into a small, weighed flask and distil to dryness. The residue will consist of nearly pure strychnine. Dry at 95 to 100° and weigh. C. C. Keller has found that 48 per cent. is the average quantity of strychnine in mixed nux vomica alkaloids. Nitric acid converts brucine into non-basic substances which are not extracted by the solvent used.

ESTIMATION OF STRYCHNINE IN MIXTURES OF STRYCHNINE AND BRUCINE.

(H. M. Gordin's[1] Method.)

Dissolve the mixed alkaloids (0.2 to 0.3 grams) upon the water-bath in 15 c.c. of 3 per cent. sulphuric acid, and add 3 c.c. of a dilute nitric acid (equal parts of nitric acid (sp. gr. 1.42) and water) to the cold solution. Pour the mixture into a separating funnel after exactly 10 minutes, and add sodium hydroxide solution in strong excess. Extract strychnine three times with chloroform. Pass the chloroform solution through a double paper into a small, weighed flask, add 2 c.c. of pure amyl alcohol and distil to dryness. Remove the last traces of liquid, which consist mainly of amyl alcohol, by forcing a current of air through the flask warmed upon the water-bath. Finally, dry the residue 2 hours at 135° and weigh.

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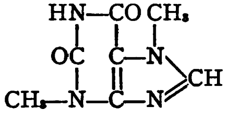
[1] *Archiv der Pharmacie*, 240, 643 (1902).

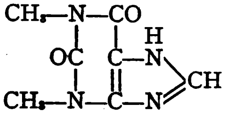
ESTIMATION OF THEOBROMINE IN CACAO-SEEDS.

1. **Kunze's Method.**—Heat 10 grams of powdered cacao-seeds 20 minutes with 150 c.c. of 5 per cent. sulphuric acid. Filter while hot and add excess of sodium phospho-molybdate to the warm filtrate. Set aside for a day and wash the precipitate upon a filter with 5 per cent. sulphuric acid. Decompose the moist precipitate, containing theobromine, in a large beaker with barium hydroxide. Precipitate excess of barium hydroxide by carbon dioxide, evaporate the mixture to dryness upon the water-bath and extract the well-dried residue under a reflux condenser with boiling chloroform. Evaporate the chloroform solution in a weighed flask and dry the residue to constant weight. This residue consists essentially of theobromine mixed with some caffeïne. This gives the weight of total alkaloids. To estimate theobromine, dissolve the residue in water containing ammonia, add excess of N/10 silver nitrate solution in known quantity and heat to boiling. This will precipitate the silver salt of theobromine ($C_7H_7N_4O_2Ag + 1\frac{1}{2}H_2O$) free from caffeïne. Determine excess of silver in an aliquot portion of the measured filtrate by titration with N/10 sulphocyanate solution. Caffeïne does not form a silver compound. To determine the corresponding quantity of theobromine, multiply the weight of silver in the precipitate by 1.66.

$$C_7H_8N_4O_2 : Ag = 180 : 108 = 1.66.$$

Theobromine is 3,7-dimethyl-xanthine and is isomeric with theophylline, 1,3-dimethyl-xanthine.


Theobromine.


Theophylline.

The latter occurs in tea-leaves.

2. Decker's Method Modified by P. Welmans.[1]—Boil 5 grams of powdered cacao-seeds which have not been freed from fat, or 10 grams of chocolate, for an hour in a large Erlenmeyer flask under a reflux condenser with 5 grams of magnesium oxide (calcined magnesia) and 300 c.c. of water. Let the flask stand upon a boiling water-bath until the suspended matter has settled and then pour the hot, supernatant liquid through an asbestos filter. Wash the residue in the flask twice with 200 c.c. portions of boiling water. Pour the clear liquid through the same asbestos filter. It is advisable to use a water-pump in filtering. Boil the residue again for an hour under a reflux condenser with 2 grams of magnesium oxide and 300 c.c. of water, decant and filter. Mix all the filtrates with ignited sea-sand and evaporate to dryness upon the water-bath. Reduce the residue in a hot mortar to as fine a powder as possible, extract 3 or 4 times in an Erlenmeyer flask for 30 minutes with 70 c.c. portions of chloroform and filter hot. Distil each portion of chloroform upon the water-bath and use the solvent again. Pass a gentle stream of air through the flask, until there is no odor of chloroform. Dissolve the residue in 10 per cent. ammonium hydroxide solution and filter. Evaporate the filtrate to dryness in a weighed platinum dish, dry the residue 30 minutes at 100° and weigh. Ignite the residue to constant weight and deduct the weight of ash from the weight of total alkaloids. Caffeïne and theobromine can be separated by cold benzene, theobromine being soluble only to the extent of 1 : 100,000, whereas caffeïne passes easily into solution.[2]

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[1] *Pharmaceutische Zeitschrift,* 47, 798 and 858 (1902).

[2] Dr. Thiel has discussed critically the different methods used in estimating theobromine in cacao preparations (*Forschungsberichte für Nahrungsmittel,* etc., I, 108 (1894)).

ESTIMATION OF ALKALOIDS IN CERTAIN OFFICINAL EXTRACTS.

ESTIMATION OF ATROPINE IN EXTRACT OF BELLADONNA.

(As Directed in the German Pharmacopoeia.)

Dissolve 2 grams of extract of belladonna in an Erlenmeyer flask in 5 grams of water and 5 grams of absolute alcohol, and add 50 grams of ether and 20 grams of chloroform to this solution. Shake vigorously and add 10 c.c. of sodium carbonate solution (1 : 3). Let the mixture stand and agitate at frequent intervals for an hour. Then pass 50 grams of the clear ether-chloroform solution through a dry filter kept well-covered, and receive the filtrate in a flask. Distil half the solvent and pour the remainder into a separating funnel. Wash the flask three times with 5 c.c. portions of ether. Thoroughly extract the total ether-chloroform solution with 20 c.c. of N/100 hydrochloric acid. When the liquids have separated clear, if necessary, after addition of enough ether to bring the ether-chloroform solution to the surface, pass the acid solution through a small filter moistened with water and receive the filtrate in a 200 c.c. flask. Make three extractions of the ether-chloroform solution with 10 c.c. portions of water, and pass these washings through the same filter. Finally, wash the filter with water and bring the entire solution to 100 c.c. Add enough ether to make a layer 1 cm. thick and 5 drops of iodeosine solution. Run in N/100 potassium hydroxide solution, shaking vigorously after each addition, until the aqueous solution has a pale red color. This should require not more than 13 c.c. of alkaline solution. The residue obtained by evaporating the ether-chloroform solution should give the atropine reactions.

Calculation.—The 20 c.c. of N/100 hydrochloric acid used is in excess of the quantity needed by the alkaloid.

Not more than 13 c.c. of N/100 potassium hydroxide solution should be required in the neutralization. Consequently, 7 c.c. of acid have combined with the alkaloid. Only 150 c.c., that is, two-thirds of the original mixture, have been used, and 10.5 c.c. of N/100 hydrochloric acid would be required to combine with the atropine in 2 grams of belladonna extract. Since 289.27 is the equivalent weight of the mon-acid base atropine, 10.5 c.c. of acid represent $10.5 \times 0.0028927 = 0.03037$ grams of atropine. In other words, extract of belladonna should contain at least 1.5187 per cent. of atropine.

Extract of Hyoscyamus.

The method of estimating alkaloid in this extract is the same as that used for extract of belladonna. Use 10 c.c. instead of 20 c.c. of N/100 hydrochloric acid for this extract. In titrating excess of acid, not more than 6.5 c.c. of N/100 potassium hydroxide solution should be required to produce the pale red color.

Employ the same calculation used for extract of belladonna. Atropine and hyoscyamine, being isomeric compounds, have the same equivalent weight. Consequently, $10 - 6.5 = 3.5$ c.c. of N/100 hydrochloric acid are required. Hence, $3.5 \times 0.0028927 = 0.0101245$ grams of alkaloid. Two-thirds of the original material have been taken. Two grams of extract of hyoscyamus should contain, therefore, at least 0.015186 grams of alkaloid which correspond to 0.7594 per cent.

Extract of Nux Vomica.

Estimate alkaloids in 1 gram of extract of nux vomica by the method used for the two preceding extracts. Instead of washing the flask three times with pure ether, use three 5 c.c. portions of a mixture of 3 parts of ether and 1 part of chloroform. Thoroughly extract the combined solutions with 50 c.c. of N/100 hydrochloric acid. "Not more than 18 c.c. of N/100 potassium hydroxide solution should be required to produce the pale red color."

Calculation.—Nux vomica contains about equal parts of strychnine and brucine, and the mean equivalent for the mixed alkaloids is

$$\frac{334 + 394}{2} = 364.$$

Since 50 — 18 = 32 c.c. of N/100 hydrochloric acid, needed to combine with the alkaloids, 32 × 0.00364 = 0.16648 grams of mixed alkaloids. But only two-thirds of the extract have been used, and this value must be increased by one-half. This gives 0.17472 grams, or extract of nux vomica should contain at least 17.472 per cent. of alkaloids, that is to say, strychnine and brucine.

TINCTURE OF NUX VOMICA.

Evaporate 50 grams of tincture of nux vomica in a weighed dish to 10 grams. Wash this residue into an Erlenmeyer flask and rinse with 5 grams of absolute alcohol. Add 50 grams of ether and 20 grams of chloroform and shake vigorously. Then add 10 c.c. of sodium carbonate solution (1 : 3) which has been previously employed in rinsing the dish used in evaporating the tincture. Let this mixture stand an hour, shaking vigorously at frequent intervals. Filter 50 grams of the clear ether-chloroform solution. To extract alkaloids, use 40 c.c. of N/100 hydrochloric acid. Not more than 17 c.c. of N/100 potassium hydroxide solution should be required in titrating excess of acid. In other respects, the estimation of alkaloids is the same as described for extract of nux vomica.

ESTIMATION OF ALKALOID IN POMEGRANATE BARK.

(German Pharmacopoeia.)

The bark of the stem and root of the pomegranate tree acts as a taenifuge and taenicide. This is due to the presence of several alkaloids. Tanret has isolated four distinct bases, namely, pelletierine ($C_8H_{15}NO$), iso-pelletierine ($C_8H_{15}NO$), methyl-pelletierine ($C_9H_{17}NO$) and pseudo-pelletierine ($C_9H_{15}NO$). These alkaloids are named after the French alkaloid-chemist, Pelletier. The first three are liquids, whereas pseudo-pelletierine is a solid crystallizing in prisms.

To determine total alkaloids, pour 90 grams of ether and 30 grams of chloroform upon 12 grams of rather finely ground pomegranate bark, dried at 100° and placed in an Erlenmeyer flask. Shake vigorously and add 10 c.c. of a mixture of 2 parts of sodium hydroxide solution and 1 part of water. Let the mixture stand 3 hours, shaking vigorously at frequent intervals. Then add 10 c.c. of water, which will cause the powder to gather into balls after vigorous shaking, and leave the supernatant ether-chloroform solution perfectly clear. After an hour, pass 100 grams of the clear ether-chloroform solution through a dry filter, kept well-covered,* and receive the filtrate in a separating funnel. Extract this filtrate with 50 c.c. of N/100 hydrochloric acid and pass this acid solution, when perfectly clear, through a small filter moistened with water into a 100 c.c. flask. Make three more extractions with 10 c.c. portions of water, and pass these extracts through the same filter. Wash the filter with water and dilute the total solution with water to 100 c.c. Place 50 c.c. of this solution in a 200 c.c. flask with 50 c.c. of water and enough ether to make a layer about 1 cm. thick. Add 5 drops of iodeosine solution and enough N/100 potassium hydroxide solution, shaking vigorously after each addition, to give a pale red color to the aqueous solution. This should require not more than 11 c.c. of alkaline solution.

ESTIMATION OF ALKALOID IN ACONITE ROOT.

Several alkaloids have been isolated from different members of the aconite family, but very little is known as yet regarding their chemical constitution. The officinal Aconitum Napellus contains the two bases, aconitine ($C_{33}H_{45}NO_{12}$?) and picraconitine ($C_{31}H_{39}NO_7(OH)_4$), which in the plant are combined with aconitic acid. Aconitine crystallizes in prisms, which are almost insoluble in water, easily soluble in benzene, alcohol and chloroform and soluble with difficulty in ether. It has an acrid and very bitter taste, and is one of the most poisonous of the alkaloids. Aconitine is a weak, tertiary base, containing four methoxyl-groups and probably three hydroxyl-groups.

Total alkaloids in aconite root are estimated very nearly according to the method used for pomegranate bark. From the point marked by an asterisk in the preceding process, the German Pharmacopoeia directs as follows: " . . . and receive the filtrate in a small flask. Distil about half the solvent and pour the remainder into a separating funnel. Wash the flask with three 5 c.c. portions of a mixture of 3 parts of ether and 1 part of chloroform, and thoroughly shake the combined solutions with 25 c.c. of N/100 hydrochloric acid. When the liquids have separated perfectly clear, add enough ether to bring the ether-chloroform solution to the surface. Pass the acid solution through a small filter moistened with water and collect the filtrate in a 100 c.c. flask. Make three more extractions of the ether-chloroform solution with 10 c.c. portions of water, and pass these extracts through the same filter. Wash the latter with water and dilute the total solution with water to 100 c.c. Place 50 c.c. of this solution in a 200 c.c. flask with 50 c.c. of water and enough ether to make a layer about 1 cm. thick. Add 5 drops of iodeosine solution and run in, while shaking, enough N/100 potassium hydroxide solution to give the aqueous solution a pale red color. This should require not more than 8.5 c.c. of alkaline solution." Upon the basis of 647 for the molecular weight of aconitine ($C_{33}H_{45}NO_{12}$), the German Pharmacopoeia requires at least 0.516 per cent. of aconitine in aconite root.

APPENDIX.

A. GENERAL ALKALOIDAL REAGENTS.

The so-called "General Alkaloidal Reagents," when added to solutions of most of the free alkaloids or of their salts, produce precipitates. These are characterized by their color, their amorphous or crystalline appearance and their insolubility or sparing solubility in water. These reagents, however, do not precipitate alkaloids exclusively. Several of them, for example, the chlorides of gold, platinum and mercury, phospho-molybdic and phospho-tungstic acids, react similarly with ammonia and many ammonium derivatives. This is not surprising, since the alkaloids, which are usually secondary or tertiary bases, are themselves ammonium derivatives. Most of the general, alkaloidal reagents also precipitate albumin, albumoses, peptones, creatinine and the nuclein-bases, adenine, guanine, hypoxanthine and xanthine.

Consequently, the general, alkaloidal reagents are especially useful in detecting the presence of alkaloids or other basic compounds. If the ether extract of the alkaline solution leave only a slight residue, this should first be examined with the general, alkaloidal reagents and then tested, if necessary, for individual alkaloids. These tests may be made with a solution prepared by dissolving the alkaloid, that is to say, the residue from the ether extract of the alkaline solution in the Stas-Otto method, in water containing a trace of hydrochloric or sulphuric acid. The general, alkaloidal reagents usually react also with aqueous solutions of the easily soluble, free alkaloids.

The most important general, alkaloidal reagents are the following:

Gold Chloride.—An aqueous solution (1:30) of $HAuCl_4.3H_2O$. It produces white, yellow or brown precipitates which are amorphous or crystalline. These precipitates decompose, to some extent, with separation of metallic gold.

Platinum Chloride.—An aqueous solution (1:20) of $H_2PtCl_6.6H_2O$. It produces yellowish-white to yellow precipitates which are usually granular and crystalline. These precipitates are double salts, analogous to the compound formed by this reagent with ammonium chloride.

Mercuric Chloride.—An aqueous solution (1:20) of $HgCl_2$. It produces white or yellowish precipitates which are usually amorphous, though they may gradually become crystalline.

Iodine Solution, Iodine-Potassium Iodide.—(Bouchardat's Reagent.) A solution of 1 part of iodine and 2 parts of potassium iodide in 20 parts of water. It produces brown precipitates which are usually flocculent.

Potassium Cadmium Iodide.—(Marmé's Reagent.) Dissolve 20 grams of potassium iodide in the same quantity of boiling water and add 10 grams of cadmium iodide. Dilute this solution to 100 c.c. Marmé's reagent produces white or yellowish precipitates with sulphuric acid solutions of most of the alkaloids, even when the solutions are very dilute. These precipitates, at first amorphous, become crystalline later. They are soluble in excess of the reagent and also in alcohol.

Potassium Mercuric Iodide.—(Mayer's Reagent.) A solution of 1.35 grams of mercuric chloride and 5 grams of potassium iodide in 100 grams of water. It produces white, or amorphous precipitates, with hydrochloric acid solutions of most of the alkaloids. These precipitates, at first amorphous, gradually become crystalline.

Potassium Bismuthous Iodide.—(Dragendorff's Reagent.) Dissolve bismuthous iodide (BiI_3) in a hot, concentrated, aqueous solution of potassium iodide. Add the same volume of potassium iodide solution. The reagent may also be prepared by suspending 1.5 grams of bismuth subnitrate in 20 c.c. of water and heating to boiling. Then add 7 grams of potassium iodide and finally 20 drops of dilute hydrochloric acid. This reagent produces orange-red precipitates with sulphuric acid solutions of many alkaloids. These precipitates are usually amorphous.

Phospho-Molybdic Acid.—(Sonnenschein's Reagent.) (*a*) Saturate sodium carbonate solution with pure molybdic acid. Add 1 part of crystallized disodium phosphate ($Na_2HPO_4.12H_2O$) to 5 parts of the acid and evaporate to dryness. Fuse the residue in a porcelain crucible and dissolve the cold melt in water. Prepare 10 parts of solution from 1 part of this residue. Add enough nitric acid to the filtered solution to produce a golden-yellow color.

(*b*) When molybdic acid is not obtainable, completely precipitate at 40° with excess of sodium phosphate solution the nitric acid solution of ammonium molybdate, used in testing for phosphoric acid. Thoroughly wash the yellow precipitate, add water and dissolve in warm sodium carbonate solution. Evaporate this solution to dryness and fuse the residue until ammonia is completely expelled. If there has been any reduction, which is indicated by a blue or black color, moisten the residue with nitric acid and fuse again. Dissolve this residue in hot water and add a large excess of nitric acid. Prepare 10 parts of solution from 1 part of residue. The golden-yellow solution must be protected from ammonia vapor.

Phospho-molybdic acid produces yellowish, amorphous precipitates with sulphuric acid solutions of most of the alkaloids. After some time, these precipitates frequently have a greenish or bluish color, due to reduction of molybdic acid to molybdic oxide.

Phospho-Tungstic Acid.—(Scheibler's Reagent.) Add a small quantity of 20 per cent. phosphoric acid to an aqueous solution of sodium tungstate. This reagent produces precipitates similar to those given by the preceding reagent.

Tannic Acid.—A 5 per cent. aqueous solution of tannin. It produces whitish or yellowish, flocculent precipitates which are perfectly soluble in hydrochloric acid. Alkaloids can be recovered in part from these tannic acid precipitates by treating them with lead or zinc carbonate, evaporating to dryness and extracting the residue with ether, alcohol or chloroform.

Picric Acid.—A concentrated, aqueous solution of picric acid. It produces yellow, crystalline or amorphous precipitates which soon become crystalline.

B. Other Reagents and Solutions.

Erdmann's and Froehde's reagents are two special reagents for alkaloids.

Erdmann's Reagent.—This is sulphuric acid containing nitric acid. Add 10 drops of a solution of 6 drops of concentrated nitric acid in 100 c.c. of water to 20 c.c. of pure, concentrated sulphuric acid.

Froehde's Reagent.—This is a solution of molybdic acid in sulphuric acid. Dissolve 5 milligrams of molybdic acid, or sodium molybdate, in 1 c.c. of hot, concentrated, pure sulphuric acid. This solution should be colorless. It does not keep long.

Concentrated Froehde's reagent contains 0.01 grams of molybdic acid, or its sodium salt, to 1 c.c. of concentrated sulphuric acid.

Fehling's Solution.—This reagent consists of copper sulphate solution and alkaline Rochelle salt solution. The two solutions should be kept separate.

1. **Copper Sulphate Solution.**—Dissolve 34.64 grams of pure, crystallized copper sulphate ($CuSO_4.5H_2O$) in 500 c.c. of water.

2. **Alkaline Rochelle Salt Solution.**—Dissolve 173 grams of Rochelle sale ($K.Na.C_4H_4O_6.4H_2O$) and 50 grams of sodium hydroxide in sticks in hot water, and dilute this solution when cold to 500 c.c.

These two solutions, mixed volume for volume, constitute Fehling's solution, which should be prepared just before it is to be used. Fehling's solution, which has been made up and kept, should always be tested before being used. It should be rejected, if it give a precipitate of cuprous oxide when warmed by itself.

Formaldehyde-Sulphuric Acid.—Add 2 or 3 drops of aqueous formaldehyde solution (formalin) to 3 c.c. of concentrated sulphuric acid just before using.

Günzburg's Reagent,[1] Phloroglucinol-Vanillin Solution.—Dissolve 1 part of phloroglucinol and 1 part of vanillin in 30 parts of alcohol. This reagent is used to detect free mineral acid, especially hydrochloric acid. Free organic acids do not react with it.

Hünefeld's Solution.—Add 25 c.c. of alcohol, 5 c.c. of chloroform and 1.5 c.c. of glacial acetic acid to 15 c.c. of old turpentine which has been exposed some time to air and light. It should not, however, produce a blue color with guaiac tincture direct, or with 15 c.c. of 3 to 5 per cent. acid-free hydrogen peroxide. This solution is used in the detection of blood.

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[1] Better results are obtained by preparing this reagent just before it is to be used. Keep two separate alcoholic solutions (1:15) of phloroglucinol and vanillin, and mix them volume for volume as needed. Tr.

Iodic Acid Solution.—An aqueous solution (1:10) of iodic acid (HIO_3).

Magnesia Mixture.—Dissolve 11 grams of crystallized magnesium chloride ($MgCl_2.6H_2O$) and 14 grams of ammonium chloride in 130 grams of water, and add 70 grams of ammonium hydroxide solution (sp. gr. 0.96 = 10 per cent. of NH_3). This mixture should be clear. It is used to detect arsenic acid and phosphoric acid.

Millon's Reagent.—Dissolve 1 part of mercury in 1 part of cold, fuming nitric acid. Dilute with twice the volume of water and decant the clear solution after several hours.

Nessler's Reagent.—Dissolve separately in the cold 3.5 grams of potassium iodide in 10 c.c. of water, and 1.7 parts of mercuric chloride in 30 c.c. of water. Add mercuric chloride solution to potassium iodide solution, until there is a permanent precipitate. Dilute with 20 per cent. sodium hydroxide solution until the volume is 100 c.c. Add mercuric chloride solution, until there is again a permanent precipitate, and let the solution settle. Decant the clear solution and keep in small bottles in the dark. This reagent improves upon keeping.

Stannous Chloride Solution.—Mix 5 parts of crystallized stannous chloride with 1 part of hydrochloric acid, and saturate completely with dry hydrochloric acid gas. Let this solution settle and filter through asbestos. It is a pale, yellowish, refractive liquid (sp. gr. at least 1.9). This solution is used to detect arsenic (Bettendorff's Arsenic Test).

C. Outline of a Laboratory Course.

To become familiar with the principles of toxicological analysis and to overcome the difficulties in detecting small quantities of poisons mixed with much organic matter, the student should learn to apply the methods of analysis to unknown mixtures of poisons with vegetable or animal material. But it is not easy to lead a large class through such work. A better scheme perhaps is to limit these more difficult procedures to small classes of advanced students who are more expert in laboratory methods and who take up the work because of a desire to learn the subject.

The beginner, knowing little of the care needed in certain tests, especially those depending upon color changes, and being unfamiliar with the tests as applied even to pure substances, may spend much time in the tedious separation of a poison and, failing finally to get positive results, conclude that the methods are inadequate merely because he has not taken sufficient care in the purification of material. For this reason it would seem more logical to become acquainted first with the tests as applied to pure materials. The work outlined in this appendix is based upon this idea and the directions given are intended to aid the instructor as well as the student.

The numbers in parentheses refer to pages in the text. A laboratory period is supposed to require two or at most three hours. As an aid to the instructor, or to his assistant, full directions for the preparation of materials and reagents are given with each laboratory period. A careful study of the text should be required as a supplement to laboratory work and the student should not undertake his experimental work until he has become familiar with the pages assigned for study. Accompanying the work of each laboratory period are questions, the answers to which will be found either in the text or in the lectures given by the instructor. The suggestion is made that the instructor require the student to submit for examination written answers to these questions or to others he may choose to give.

FIRST LABORATORY PERIOD.

The work of this period will cover pages 3 to 31 (inclusive). The instructor should use his judgment in presenting on the lecture-table such tests as Mitscherlich's (4–6), Blondlot's (10–12), or others, which may seem to him inappropriate for the laboratory. The materials used in the tests selected represent the distillates mentioned on pages 6 and 14.

MATERIALS.

The quantities designated will be found ample for a class of 40 or 50 students. The student is advised to hand in beforehand four bottles for the solutions to be examined.

Phosphorus Water.—Put 2000 c.c. of distilled water into each of two glass-stoppered bottles and add a few small pieces of yellow phosphorus previously scraped under water. This should be done several days before the laboratory period and the bottles should be shaken frequently. Each student should receive 50 c.c. of this solution which will give all the phosphorus tests.

Hydrocyanic Acid.—Put 2000 c.c. of distilled water into each of two glass-stoppered bottles and dissolve in each 10 grams of potassium cyanide. Add to each bottle 15 grams of crystallized tartaric acid dissolved in the smallest possible quantity of distilled water. Shake well but *do not smell!!* This solution should be prepared the day before the laboratory period and each student should receive 50 c.c. of solution decanted from any precipitate of acid potassium tartrate. This solution will contain about 0.2 per cent. of hydrocyanic acid.

Carbolic Acid.—Put 2000 c.c. of distilled water into each of two glass-stoppered bottles and add to each 40 grams of crystallized carbolic acid. Shake frequently to saturate the water. Each student should receive 50 c.c. of this solution decanted from any undissolved carbolic acid.

Chloroform Water.—Put 2000 c.c. of distilled water into each of two glass-stoppered bottles and add to each

40 c.c. of chloroform. Shake frequently to saturate the water. Each student should receive 50 c.c. of this solution decanted from any undissolved chloroform.

REAGENTS.

All reagents required for the specified tests have been given. Laboratories of biological chemistry usually have many of these reagents on each working-desk or on the end-desks. The instructor should follow his own wishes in placing certain special reagents, which are expensive or seldom used, on the end-desks or in issuing them from the store-room. Each reagent is designated according to the above classification. If the directions seem needlessly full, they have been made so from a wish to lighten the instructor's duties by making it possible for an assistant, who is often inexperienced, to attend to this part of the work.

1. **Chlorine Water.**—This solution, which should be fresh, is easily made by filling a non-tubulated, glass retort with distilled water, inverting and removing water until just enough remains to keep the body of the retort full. Pass chlorine gas through a glass tube into the retort until the water is saturated. *End-desk.*

2. **Concentrated Nitric Acid.**—A chemically pure strong acid having a strength of about 70 per cent. *Working-desk.*

3. **Ammonium Molybdate.**—Dissolve 1 part of molybdic acid in 4 parts of strong ammonia, taking care to add only a little molybdic acid at a time and to stir after each addition. Filter the ammoniacal solution, and allow it to cool. Mix the clear solution with 15 parts of strong nitric acid, adding the ammonia solution slowly, with constant stirring, taking care to keep the mixture cool. Let the mixture stand 24 hours in a warm place and filter; or dissolve 1 part of ammonium molybdate in 3 or 4 parts of weak ammonia, and mix the liquid with 12 or 15 parts of nitric acid as before. *End-desk.*

4. **Magnesia Mixture.**—Prepare this reagent as directed on page 217. *End-desk.*

5. **Silver Nitrate.**—Dissolve 1 part by weight of the crystallized salt in 20 parts of water. *End-desk.*

6. **Lead Acetate.**—Dissolve 1 part by weight of the crystallized salt in 10 parts of water and filter. *End-desk.*

7. **Hydrogen Sulphide Water.**—Saturate distilled water with hydrogen sulphide gas in the apparatus used in preparing chlorine water. *Special reagent.*

8. **Tartaric Acid.**—Dissolve 1 part by weight of the crystallized acid in 20 parts of water. *Special reagent.*

9. **Guaiac Paper.**—Prepare this paper as directed in the foot-note on page 17. *Special reagent.*

10. **Copper Sulphate.**—Dissolve 1 part by weight of the crystallized salt in 1000 parts of water; or dilute 1 c.c. of the ordinary solution (1 : 10) to 100 c.c. *Special reagent.*

11. **Potassium or Sodium Hydroxide.**—Dissolve 1 part by weight of the caustic alkali in 10 parts of water. Allow the solution to stand and siphon the clear liquid from any sediment. *Working-desk.*

12. **Ferrous Sulphate.**—Instead of using ferrous sulphate, solutions of which rapidly undergo oxidation, dissolve 1 part by weight of crystallized ammonium ferrous sulphate ($(H_4N)_2SO_4.FeSO_4.6H_2O$) in 20 parts of water. *Special reagent.*

13. **Ferric Chloride.**—Dissolve 1 part by weight of the salt in 15 parts of water. *End-desk.*

14. **Dilute Hydrochloric Acid.**—Mix 1 volume of pure concentrated hydrochloric acid with 4 volumes of water. *Working-desk.*

15. **Yellow Ammonium Sulphide.**—Dissolve sulphur to saturation in freshly prepared ammonium sulphide, or use an ammonium sulphide solution which has become yellow from standing. *End-desk.*

16. **Potassium or Sodium Nitrite.**—Dissolve 1 part by

weight of the fused or crystallized commercial salt in 20 parts of water. *Special reagent.*

17. **Dilute Sulphuric Acid.**—Mix 1 volume of pure concentrated sulphuric acid with 4 volumes of water, pouring the acid slowly into the water and stirring constantly. *Working-desk.*

18. **Ammonium Hydroxide.**—Dilute 1 volume of strong ammonia, which is usually about 20 per cent., with 3 volumes of water. *Working-desk.*

19. **Ammonium Sulphide.**—Pass hydrogen sulphide gas through ammonium hydroxide solution, which has the same strength as that used on the working-desk, until a portion of the liquid yields no precipitate when tested with a drop of a solution of magnesium sulphate. This is the ordinary solution used in the laboratory and contains approximately 7.5 per cent. of ammonium sulphide.

To prepare a 1 per cent. solution, which is the reagent used in the present instance, dilute 3 volumes of the above solution with 17 volumes of water. *Special reagent.*

20. **Dilute Nitric Acid.**—Mix 1 volume of pure concentrated acid with 5 volumes of water. *Working-desk.*

21. **Picric Acid.**—Dissolve 1.2 parts by weight of the crystallized acid in 100 parts of water. This will give a saturated solution which may be filtered or decanted in case a portion of the acid is undissolved. *End-desk.*

22. **Millon's Reagent.**—Prepare this reagent as directed on page 217. *End-desk.*

23. **Bromine Water.**—Fill a glass-stoppered bottle with water and pour into it a few drops of bromine and shake the bottle frequently. There should be an excess of bromine at the bottom of the bottle from which the solution should be decanted. *End-desk.*

24. **Calcium Hypochlorite.**—Grind 100 grams of bleaching powder, which should smell strongly of chlorine, in a mortar, using in all 1000 c.c. Start the day before the

laboratory period and shake the mixture frequently. Finally filter and use the clear filtrate. *Special reagent.*

25. **Benzaldehyde.**—Use the commercial benzaldehyde which is a yellowish oil with an agreeable odor of bitter almonds. *Special reagent.*

26. **Aniline Hydrochloride.**—Dissolve 10 grams of aniline in 1000 c.c. of water. If the oil does not dissolve completely, add 1 c.c. of concentrated hydrochloric acid at a time, shaking after each addition, until the solution is clear. *Special reagent.*

27. **Fehling's Solution.**—Prepare this reagent as directed on page 215. *End-desk.*

28. **Resorcinol Solution.**—Dissolve 1 part by weight of crystallized resorcinol in 20 parts of water. *Special reagent.*

29. **Alkaline α-Naphthol Solution.**—Dissolve 1 part by weight of crystallized α-naphthol in 40 c.c. of 33 per cent. sodium hydroxide solution. *Special reagent.*

EXPERIMENTAL WORK.

Phosphorus.—Apply the tests designated, using the phosphorus water supplied. Note the odor of the solution.

1. Mix in a porcelain dish 40 c.c. of the phosphorus solution with the same volume of chlorine water. What action has chlorine water upon phosphorus? Set the dish on an iron gauze, evaporate nearly to dryness with a free flame and complete the evaporation on the water-bath. Dissolve the residue in 5 c.c. of water and divide the solution into two equal portions. Apply the ammonium molybdate and the ammonium magnesium phosphate tests (6).

2. Into two separate Erlenmeyer flasks put 10 c.c. of phosphorus water and 10 c.c. of hydrogen sulphide water. Apply Scherer's test to each, using lead acetate and silver nitrate papers. Compare the results (6).

Hydrocyanic Acid.—Apply the tests designated, using the hydrocyanic acid supplied. Owing to the exceedingly poisonous properties of this acid, use the greatest care in manipulation.

1. *Schönbein's Test.*—Put 5 c.c. of the solution into a small Erlenmeyer flask. If the solution does not show an acid reaction with blue litmus paper, add 5 c.c. of tartaric acid solution. Suspend guaiac paper, previously moistened with dilute copper sulphate solution, in the neck of the flask. Warm the solution gently on the water-bath and stop as soon as the test appears (17).

2. *Prussian Blue Test.*—Use 5 c.c. of the solution for this test (18).

3. *Sulphocyanate Test.*—Use 5 c.c. of the solution for this test (18).

4. *Vortmann's Test.*—Use 5 c.c. of the solution for this test (19).

5. *Silver Nitrate Test.*—Use 5 c.c. of the solution for this test (19).

6. *Picric Acid Test.*—Use 5 c.c. of the solution for this test (20).

Carbolic Acid.—Apply the tests designated, using the carbolic acid solution supplied. Note the odor of the solution.

1. *Millon's Test.*—Use 5 c.c. of the solution for this test (23).

2. *Bromine Water Test.*—Use 5 c.c. of the solution for this test (24).

3. *Ferric Chloride Test.*—Use 5 c.c. of the solution for this test (24).

4. *Calcium Hypochlorite Test.*—Use 5 c.c. of the solution for this test (24).

5. *Melzer's Test.*—Use 5 c.c. of the solution for this test (25).

Chloroform.—Apply the tests designated, using the chloroform solution supplied. Note the odor and the taste of this solution.

1. *Phenyl-isocyanide Test.*—Use 5 c.c. of the solution for this test. Instead of free aniline, use a solution of aniline hydrochloride as the reagent. The alkali will set aniline free. When many students are making this test, the odor is so disagreeable that it is advisable to provide a special receptacle (bottle) into which test-tubes are emptied (29).

2. *Fehling's Test.*—Use 5 c.c. of the solution for this test (29).

3. *Silver Reduction Test.*—Use 5 c.c. of the solution for this test (29).

4. *Resorcinol Test.*—Use 5 c.c. of the solution for this test (30).

5. *Lustgarten's Test.*—Use 5 c.c. of the solution for this test (30).

QUESTIONS.

1. Name in order of importance the organs which it is best to take for the purpose of making a toxicological analysis.

2. Describe a method of transmitting organs after their removal from the body.

3. When organs must be kept for some time, what can you say as to the use of preservatives?

4. Why is it improper to embalm a body in case of suspected poisoning?

5. Into how many groups may poisons be divided and what are some of the characteristics of each group?

6. Describe in full the method of procedure in distilling for volatile poisons.

7. Name some of the more important substances found in the group of volatile poisons.

8. What preliminary test should be made for phosphorus before beginning the distillation?

9. How might processes of oxidation affect the tests for phosphorus which depend upon the volatility of this element?

10. Describe the Mitscherlich test and state what may prevent a positive result.

11. Describe Schönbein's test for hydrocyanic acid and state what conclusions can be drawn from it. How may hydrocyanic acid be identified positively?

12. How may carbolic acid be identified positively? What effect has the internal administration of this substance upon the urine?

SECOND LABORATORY PERIOD.

The work of this period will cover pages 31 to 48 (inclusive). The materials used in the tests selected represent the distillates mentioned on pages 14 to 16.

MATERIALS.

The student is advised to hand in beforehand seven bottles for the solutions to be examined.

Chloral Hydrate.—Put 2000 c.c. of distilled water into each of two glass-stoppered bottles and add to each 40 grams of crystallized chloral hydrate. Shake thoroughly and supply each student with 50 c.c. of this 2 per cent. solution.

Iodoform.—Dissolve 10 grams of crystallized or precipitated iodoform in 500 c.c. of strong alcohol. Supply each student with 10 c.c. of this 2 per cent. solution.

Nitrobenzene.—Dissolve 10 grams of commercial nitrobenzene in 500 c.c. of strong alcohol. Supply each student with 10 c.c. of this 2 per cent. solution.

Aniline.—Put 2000 c.c. of distilled water into each of two glass-stoppered bottles and add to each 40 grams of commercial aniline oil. If the two liquids be thoroughly shaken, the aniline will be dissolved. Supply each student with 50 c.c. of this 2 per cent. solution.

Ethyl Alcohol.—Mix thoroughly 250 c.c. of strong alcohol with 4000 c.c. of water. Supply each student with 50 c.c. of this solution.

Acetone.—Mix thoroughly 250 c.c. of commercial acetone with 4000 c.c. of water. Supply each student with 50 c.c. of this solution.

Carbon Disulphide.—Put 2000 c.c. of distilled water into each of two glass-stoppered bottles and add to each 10 c.c. of carbon disulphide. Shake the liquids together frequently and finally decant from any undissolved carbon disulphide. Supply each student with 50 c.c. of this solution.

REAGENTS.

Since the list of reagents required for each laboratory period has been prepared especially for the convenience of the instructor, every reagent needed has been mentioned. But if the preparation of a reagent has already been described, only the page references where the description appears will follow the name of the reagent.

1. **Sodium or Potassium Hydroxide.**—See page 221.

2. **Magnesium Oxide.**—Calcined magnesium oxide. *Special reagent.*

3. **Mercuric Chloride.**—Dissolve 1 part by weight of "corrosive sublimate" in 20 parts of water. *End-desk.*

4. **Silver Nitrate.**—See page 221.

5. **Nessler's Reagent.**—Prepare this reagent as directed on page 217. *Special reagent.*

6. **Sodium Hyposulphite.**—Dissolve 1 part by weight of crystallized sodium hyposulphite in 20 parts of water. *Special reagent.*

7. **Sodium Phenolate.**—Dissolve 80 grams of solid sodium hydroxide in 150 c.c. of water. Use this solution to dissolve 40 grams of crystallized carbolic acid. *Special reagent.*

8. **Alcoholic Potassium Hydroxide.**—Dissolve 1 part by weight of potassium hydroxide in 10 parts by volume of strong alcohol. *Special reagent.*

9. **Aniline Hydrochloride.**—See page 223.

10. **Metallic Tin.**—Granulated tin which has been prepared by fusing the metal and pouring slowly into water. *Special reagent.*

11. **Concentrated Hydrochloric Acid.**—A chemically pure strong acid having a strength of about 35 per cent. *Working-desk.*

12. **Ether.**—A good commercial ether such as is used for purposes of anæsthesia. Owing to the extreme inflammability of ether, it should never be evaporated except on a water-bath and then only after all flames have been extinguished. *Special reagent.*

13. **Chloroform.**—A good commercial chloroform such as is used for purposes of anæsthesia. Chloroform may be evaporated without fear, since it is not inflammable. *Special reagent.*

14. **Calcium Hypochlorite.**—See page 222.

15. **Bromine Water.**—See page 222.

16. **Iodine Solution.**—Prepare this reagent as directed on page 213. *End-desk.*

17. **Benzoyl Chloride.**—Kahlbaum's benzoyl chloride. *Special reagent.*

18. **Dilute Sulphuric Acid.**—See page 222.

19. **Potassium Chromate.**—Dissolve 1 part by weight of the crystallized salt in 10 parts of water. *End-desk.*

20. **Potassium Hydroxide.**—Solid potassium hydroxide in rods. *Special reagent.*

21. **Carbon Disulphide.**—The commercial product. Great care should be taken in using this reagent, as it is extremely inflammable, and the vapor mixed with air is violently explosive. *Special reagent.*

22. **Ammonium Molybdate.**—See page 220.

23. **Concentrated Sulphuric Acid.**—A pure, strong acid having a sp. gr. of 1.84. *Working-desk.*

24. **Anhydrous Sodium Acetate.**—Prepared by heating crystallized sodium acetate over a free flame until all water

of crystallization has been expelled. During heating, the salt should be constantly stirred. *Special reagent.*

25. **Sodium Nitro-prusside.**—Dissolve 1 part of the crystallized salt in 20 parts of water. This reagent should be prepared the day before it is to be used and only in such quantity as is needed. *Special reagent.*

26. **Acetic Acid.**—A pure acid having a strength of about 30 per cent. *Working-desk.*

27. **Ortho-Nitro-Benzaldehyde.**—Kahlbaum's preparation. Mix 5 grams of this compound with 200 c.c. of water and heat to boiling. Cool the solution and filter. *Special reagent.*

28. **Ammonium Sulphide.**—See page 222.

29. **Lead Acetate.**—See page 221.

30. **Concentrated Ammonium Hydroxide.**—Pure ammonia water having a strength of about 20 per cent. *Special reagent.*

31. **Dilute Hydrochloric Acid.**—See page 221.

32. **Ferric Chloride.**—See page 221.

33. **Alcoholic Potassium Hydroxide.**—Add solid potassium hydroxide to absolute alcohol until it ceases to be dissolved. *Special reagent.*

34. **Copper Sulphate.**—Dissolve 1 part by weight of the crystallized salt in 10 parts of water. *End-desk.*

Experimental Work.

Chloral Hydrate.—Apply the tests designated, using the solution of chloral hydrate supplied. Note the odor and the taste of this solution.

1. Heat 5 c.c. of this solution in a test-tube with the same volume of sodium hydroxide solution. Note the odor. Chloral hydrate can be converted into chloroform by alkalies. What is the reaction? (32)

2. *Action of Magnesium Oxide.*—Put 20 c.c. of this solution into a small flask and add 1 gram of calcined mag-

nesium oxide. Close the flask with a cork through which passes a glass tube about 3 feet long. Heat the flask for 30 minutes on the water-bath. Filter, divide the filtrate into two equal portions and apply the mercuric chloride and silver nitrate tests (32).

3. *Nessler's Test.*—Use 5 c.c. of the solution for this test (33).

4. *Sodium Hyposulphite Test.*—Use 5 c.c. of the solution for this test (33).

Iodoform.—Apply the tests designated, using the alcoholic solution of iodoform supplied. Note the odor of this solution.

1. *Crystalline Form.*—Allow a drop of this solution to evaporate on a glass slide and then examine under the microscope (38).

2. *Lustgarten's Test.*—Use 3 c.c. of the solution for this test (33).

3. *Phenyl-isocyanide Test.*—Follow the directions given for chloroform (29).

Nitrobenzene.—Apply the tests designated, using the alcoholic solution of nitrobenzene supplied. Note the odor of this solution.

1. *Phenyl-isocyanide Test.*—Add two drops of chloroform to 2 c.c. of the nitrobenzene solution, then 2 c.c. of sodium hydroxide solution and warm the mixture. If the result is negative, state the reason why it is so.

2. *Reduction to Aniline.*—Put the remainder of the nitrobenzene solution into a small flask and add two pieces of metallic tin. Next add 5 c.c. of concentrated hydrochloric acid. Heat on the water-bath until the odor of nitrobenzene has disappeared. Pour the resulting solution from the tin into a test-tube and cool. Render alkaline with sodium hydroxide solution. Cool and add the same volume of ether. Cork the test-tube and shake. Pour the ether solution upon a dry filter-paper and collect the filtrate in

a dish. Evaporate the ether on the water-bath without a flame. Test the residue for aniline by means of the phenyl-isocyanide test, using chloroform and sodium hydroxide solution. What is the reaction? (36)

Aniline.—Apply the tests designated, using the solution of aniline supplied. Note the odor of this solution.

1. *Hypochlorite Test.*—Use 5 c.c. of the solution for this test (36).
2. *Bromine Water Test.*—Use 5 c.c. of the solution for this test (36).
3. *Phenyl-isocyanide Test.*—Use 5 c.c. of the solution for this test (37).

Ethyl Alcohol.—Apply the tests designated, using the solution of alcohol supplied.

1. *Lieben's Test.*—Use 5 c.c. of the solution for this test (38).
2. *Berthelot's Test.*—Use 5 c.c. of the solution for this test (38).
3. *Oxidation Test.*—Use 5 c.c. of the solution for this test (39).
4. *Vitali's Test.*—Use 5 c.c. of the solution for this test (39).
5. *Ethyl Acetate Test.*—Use 5 c.c. of the solution for this test (39).

Acetone.—Apply the tests designated, using the solution of acetone supplied.

1. *Lieben's Test.*—Use 5 c.c. of the solution for this test (40).
2. *Legal's Test.*—Use 5 c.c. of the solution for this test (41).
3. *Penzoldt's Test.*—Use 5 c.c. of the solution for this test (41).
4. *Reynold's Test.*—Use 5 c.c. of the solution for this test (41).

Carbon Disulphide.—Apply the tests designated, using the solution of carbon disulphide supplied. Note the odor of this solution.

1. *Lead Acetate Test.*—Use 5 c.c. of the solution for this test (43).

2. *Sulphocyanate Test.*—Use 5 c.c. of the solution for this test (43).

3. *Xanthogenate Test.*—Use 5 c.c. of the solution for this test (43).

QUESTIONS.

1. Describe the phenyl-isocyanide test and mention all the substances you have had which give this test.

2. How do chloroform and chloral hydrate differ as regards chemical structure? How can you distinguish one from the other by chemical tests?

3. Why does nitrobenzene fail to give the phenyl-isocyanide test? How can this test be used indirectly for the recognition of this substance?

4. Since aniline forms salts which are not volatile, how does it happen that this substance appears with the volatile poisons which are distilled from an acid solution?

5. Describe the appearance under the microscope of: (a) ammonium magnesium phosphate; (b) tribromophenol; (c) iodoform.

6. What is Lieben's test and what substances can be recognized by it?

7. What organs of the body should preferably be examined for: (a) hydrocyanic acid; (b) carbolic acid; (c) chloroform; (d) alcohol?

8. What is the structural characteristic of those substances which give positive reactions with Millon's reagent?

9. If, upon adding alcoholic sodium hydroxide solution to a liquid and applying heat, you were to get a positive phenyl-isocyanide test, what conclusions would you draw?

THIRD LABORATORY PERIOD.

The work of this period will cover pages 49 to 67 (inclusive). The materials used in the tests selected represent those non-volatile substances which are more or less completely extracted from an acid, aqueous solution by solvents immiscible with water. Some of these materials are expensive, for example, colchicin, and it is advisable to issue such substances in solution which the student may then divide into as many portions as necessary. Moreover it is desirable to accustom the student to working with small quantities in these tests.

MATERIALS.

The student is advised to hand in beforehand eight bottles for the solutions to be examined.

Picrotoxin.—Dissolve 8 grams of picrotoxin in 400 c.c. of alcohol. Supply each student with 5 to 10 c.c. of this solution.

Colchicin.—Dissolve 4 grams of colchicin in 400 c.c. of water. Supply each student with 5 to 10 c.c. of this solution.

Picric Acid.—Put 1500 c.c. of distilled water in a glass-stoppered bottle and add 20 grams of crystallized picric acid. Shake frequently and allow to stand for some time. This will give a saturated solution of picric acid. Decant from undissolved acid and supply each student with 25 c.c. of this solution.

Acetanilide.—Put 2000 c.c. of distilled water in a glass-stoppered flask and add 10 grams of crystallized acetanilide. Shake frequently and allow to stand for some time. Decant from undissolved substance and supply each student with 30 c.c. of this solution.

Phenacetine.—Dissolve 6 grams of phenacetine in 600 c.c. of alcohol and supply each student with 10 c.c. of this solution.

Antipyrine.—Dissolve 6 grams of antipyrine in 300 c.c. of water and supply each student with 5 c.c. of this solution.

Caffeïne.—Dissolve 3 grams of caffeïne in 300 c.c. of water and supply each student with 5 c.c. of this solution

Salicylic Acid.—Put 2000 c.c. of distilled water in a glass-stoppered bottle and add 5 grams of crystallized salicylic acid. Shake frequently and allow to stand for some time. Decant from undissolved substance and supply each student with 30 c.c. of this solution.

REAGENTS.

1. **Sodium Hydroxide.**—See page 221.

2. **Fehling's Solution.**—See page 216.

3. **Silver Nitrate.**—See page 221.

4. **Ammonium Hydroxide.**—See page 222.

5. **Potassium Nitrate.**—Chemically pure crystals. *End-desk.*

6. **Concentrated Sulphuric Acid.**—See page 228.

7. **Alcoholic Benzaldehyde.**—Dissolve 20 grams of benzaldehyde in 100 c.c. of absolute alcohol. *Special reagent.*

8. **Tannic Acid.**—Dissolve 1 part by weight of tannic acid in 20 parts of water. *End-desk.*

9. **Concentrated Nitric Acid.**—See page 220.

10. **Erdmann's Reagent.**—Prepare this reagent as directed on page 215. *Special reagent.*

11. **Thread.**—Woolen, cotton and silk thread. *Special reagent.*

12. **Potassium Cyanide.**—Dissolve 1 part of potassium cyanide in 2 parts of water. This will give a saturated solution. This reagent will be given out only by the instructor. *Special reagent.*

13. **Glucose.**—A good grade of commercial glucose. *Special reagent.*

14. **Concentrated Hydrochloric Acid.**—See page 228.

15. **Carbolic Acid.**—Shake distilled water with liquid carbolic acid, having the acid in excess. This will give a saturated solution. Decant from undissolved acid. *Special reagent.*

16. **Calcium Hypochlorite.**—See page 222.

17. **Chloroform.**—See page 228.

18. **Alcoholic Potassium Hydroxide.**—See page 227.

19. **Ether.**—See page 228.

20. **Chromic Acid.**—Dissolve 1 part by weight of crystallized chromic acid in 20 parts of water. *Special reagent.*

21. **Potassium or Sodium Nitrite.**—See page 221.

22. **Dilute Sulphuric Acid.**—See page 222.

23. **Ferric Chloride.**—See page 221.

24. **Chlorine Water.**—See page 220.

25. **Millon's Reagent.**—See page 217.

26. **Bromine Water.**—See page 222.

Experimental Work.

Picrotoxin.—Apply the tests designated, using the solution of picrotoxin supplied. Note the taste of this solution.

1. *Fehling's Test.*—Use one-fourth of the solution for this test (54).

2. *Silver Nitrate Test.*—Use one-fourth of the solution for this test (55).

3. *Langley's Test.*—Evaporate one-fourth of the solution on a watch-glass and test the residue (55).

4. *Melzer's Test.*—Evaporate one-fourth of the solution on a watch-glass and test the residue (55).

Colchicin.—Apply the tests designated, using the solution of colchicin supplied. Note the taste of this solution.

1. *Tannic Acid Test.*—Use one-fourth of the solution for this test. Dilute the solution with the same volume of water (56).

2. H_2SO_4-HNO_3 *Test.*—Evaporate one-fourth of the solution on a watch-glass and test the residue (56).

3. *Nitric Acid Test.*—Evaporate one-fourth of the solution on a watch glass and test the residue (57).

4. *Erdmann's Test.*—Evaporate one-fourth of the solution on a watch-glass and test the residue (57).

Picric Acid.—Apply the tests designated, using the solution of picric acid supplied. Note the taste of this solution.

1. *Dyeing Test.*—Put wool, silk and cotton into a test-tube. Allow these fibers to remain in the solution for 24 hours and then proceed as directed (58).

2. *Isopurpuric Acid Test.*—Get the saturated solution of potassium cyanide from the instructor and proceed as directed (58).

3. *Picraminic Acid Test.*—Use a fragment of glucose about the size of a rice-grain. Dissolve this in a small quantity of water and proceed as directed (58).

4. *Ammoniacal $CuSO_4$ Test.*—Prepare a small quantity of the reagent by adding ammonium hydroxide to copper sulphate solution until the precipitate at first formed is entirely dissolved and proceed as directed (58).

Acetanilide.—Apply the tests designated, using the solution of acetanilide supplied.

1. *Indophenol Test.*—Evaporate 10 c.c. of the solution in a dish, transfer the residue to a test-tube and proceed as directed (59).

2. *Phenyl-isocyanide Test.*—Render 10 c.c. of the solution alkaline with sodium hydroxide solution and boil. Proceed as directed and explain the reaction (60).

3. *Calcium Hypochlorite Test.*—Evaporate 10 c.c. of the solution in a dish and proceed as directed (60).

Phenacetine.—Evaporate all of the solution supplied, divide the residue into three equal portions and apply the tests designated.

1. *Chromic Acid Test.*—Use one-third of the residue for this test (61).

2. *Conc. HNO_3 Test.*—Use one-third of the residue and make the test on a watch-glass (62).

3. *Indophenol Test.*—Use one-third of the residue for this test (62).

Antipyrine.—Apply the tests designated, using the solution of antipyrine supplied.

1. *Nitrite Test.*—Use one-half of the solution for this test (63).

2. *Ferric Chloride Test.*—Use one-half of the solution for this test (63).

Caffeïne.—Apply the tests designated, using the solution of caffeïne supplied.

1. *Tannic Acid Test.*—Use one-half of the solution for this test (65).

2. *Oxidation Test.*—Evaporate one-half of the solution in a dish on the water-bath and test the residue as directed (65).

Salicylic Acid.—Apply the tests designated, using the solution of salicylic acid supplied.

1. *Ferric Chloride Test.*—Use 10 c.c. of the solution for this test (66).

2. *Millon's Test.*—Use 10 c.c. of the solution for this test (66).

3. *Bromine Water Test.*—Use 10 c.c. of the solution for this test (66).

QUESTIONS.

1. Describe the preliminary procedure in the "Stas-Otto" process and state what it accomplishes. What is a reflux-cooler?

2. Describe the extraction of the acid solution with ether and state what it accomplishes. What is a separating-funnel?

3. What substances are not extracted by ether owing to the presence of acid?

4. What conclusions, based on appearance, color and

taste, can be drawn from the residue of the ether extraction of the acid solution?

5. State the source and describe the characteristics of picrotoxin.

6. What substances thus far have given a positive result with Fehling's solution?

7. What is the chemical structure of picric acid? Is it a true organic acid? How are picric acid and picraminic acid related?

8. What is the chemical structure of acetanilide?

9. What substances give the indophenol test?

10. Why will not acetanilide itself give the phenyl-isocyanide test?

11. How is acetanilide altered in the organism and how may it be detected in the urine?

12. What is the chemical structure of phenacetine? How may it be distinguished from acetanilide and antipyrine?

13. What is the chemical structure of antipyrine?

14. How is caffeïne related structurally to purine?

15. What is the effect of caffeïne in the organism?

16. What is the chemical structure of salicylic acid?

17. How may salicylic acid be recognized in presence of phenols and cresols?

18. When a substance gives a positive result with Millon's reagent, what is known about its structure?

19. What is the fate of salicylic acid in the organism and how may its presence in the urine be recognized?

Fourth Laboratory Period.

The work of this period will cover pages 68 to 116 (inclusive). The materials used in the tests selected represent those non-volatile substances which are not extracted from an acid, aqueous solution by solvents immiscible with water but are extracted when the solution has been made alkaline. All of these substances here studied are vegetable alkaloids and they are of especial importance because they are much used in medicine and most of them are highly toxic in their action. As previously stated, it is advisable on account of the cost of these materials to oblige the student to work with small quantities.

MATERIALS.

The student is advised to hand in beforehand eight test-tubes which have been carefully cleaned and thoroughly dried. Since the solutions of these alkaloids are made with volatile solvents, these test-tubes should be stoppered.

Veratrine.—Dissolve 2 parts by weight of commercial veratrine in 400 c.c. of strong alcohol. Supply each student with 10 c.c. of this solution.

Strychnine.—Dissolve 1 part by weight of the free alkaloid* in 400 c.c. of chloroform. Supply each student with 10 c.c. of this solution.

Brucine.—Dissolve 1 part by weight of the free alkaloid in 200 c.c. of strong alcohol. Supply each student with 5 c.c. of this solution.

Atropine.—Dissolve 1 part by weight of the free alkaloid in 200 c.c. of chloroform. Supply each student with 5 c.c. of this solution.

Cocaïne.—Dissolve 1 part by weight of the free alkaloid in 200 c.c. of strong alcohol. Supply each student with 5 c.c. of this solution.

Codeïne.—Dissolve 2 parts by weight of the free alkaloid in 400 c.c. of strong alcohol. Supply each student with 10 c.c. of this solution.

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* The free alkaloids in these tests are to be preferred to their salts, because they are easily soluble in most cases in organic solvents and the rapid evaporation of such solutions greatly saves time. Should the free alkaloid not be accessible, dissolve an equivalent weight of one of its salts in water and after proper neutralization extract with the required volume of some immiscible solvent. Separate the two liquids and use the solution thus prepared.

Quinine.—Dissolve 2 parts by weight of the free alkaloid in 400 c.c. of chloroform. Supply each student with 10 c.c. of this solution.

Morphine.—Dissolve 2 parts by weight of morphine sulphate in 400 c.c. of water. Supply each student with 10 c.c. of this solution.

REAGENTS.

1. **Concentrated Sulphuric Acid.**—See page 228.

2. **Concentrated Hydrochloric Acid.**—See page 228.

3. **Furfurol.**—Dissolve 1 c.c. of furfurol in 100 c.c. of water. This solution should be freshly prepared. *Special reagent.*

4. **Fuming Nitric Acid.**—Use the commercial fuming acid, or better prepare a fuming acid by distilling from a glass retort equal parts by weight of dry potassium nitrate and pure concentrated sulphuric acid. *Special reagent.*

5. **Alcoholic Potassium Hydroxide.**—See page 227.

6. **Potassium Dichromate.**—The pure crystallized salt in small crystals. *Special reagent.*

7. **Tannic Acid.**—See page 234.

8. **Picric Acid.**—See page 222.

9. **Iodine Solution.**—See page 213.

10. **Potassium-Mercuric Iodide.**—Prepare this reagent as directed on page 213. *End-desk.*

11. **Concentrated Nitric Acid.**—See page 220.

12. **Stannous Chloride.**—Dissolve 1 part by weight of crystallized stannous chloride in 9 parts by weight of hydrochloric acid having a specific gravity of 1.12 (about 24 per cent. HCl). *End-desk.*

13. **Chloral Hydrate Solution.**—Dissolve 6 parts by weight of crystallized chloral hydrate in 4 parts of water. This 60 per cent. solution is used in dissolving alkaloids. *Special reagent.*

14. **Dilute Nitric Acid.**—See page 222.

15. **Dilute Hydrochloric Acid.**—See page 221.

16. **Resorcinol Solution.**—Dissolve 1 part by weight of pure resorcinol in 20 c.c. of concentrated sulphuric acid. *Special reagent.*

17. **Sodium Hydroxide.**—See page 221.

18. **$FeCl_3$-Sulphuric Acid.**—Mix 10 drops of ferric chloride solution with 100 c.c. of concentrated sulphuric acid. *Special reagent.*

19. **Froehde's Reagent.**—Prepare this reagent as directed on page 215. *Special reagent.*

20. **Dilute Sulphuric Acid.**—See page 222.

21. **Acetic Acid.**—See page 229.

22. **Chlorine Water.**—See page 220.

23. **Ammonium Hydroxide.**—See page 222.

24. **Absolute Alcohol.**—Strong alcohol dehydrated by distillation over lime and having a specific gravity of 0.7946. *Special reagent.*

25. **Alcoholic Iodine Solution.**—Dissolve 1 part by weight of iodine in 100 c.c. of strong alcohol. *Special reagent.*

26. **Formaldehyde-H_2SO_4.**—Mix 3 c.c. of 40 per cent. formaldehyde solution with 100 c.c. of concentrated sulphuric acid. *Special reagent.*

27. **Iodic Acid.**—Prepare this reagent as directed on page 217. *Special reagent.*

28. **Chloroform.**—See page 228.

29. **Neutral Ferric Chloride.**—Add sodium carbonate solution to ordinary ferric chloride solution until it causes slight turbidity. Filter and use the clear filtrate. *Special reagent.*

EXPERIMENTAL WORK.

Veratrine.—Evaporate in a dish to dryness on the water-bath the alcoholic veratrine solution. Divide the residue into four equal portions and apply the tests designated.

1. *Conc. H_2SO_4 Test.*—Apply this test to one-fourth of the residue on a watch-glass (75).

2. *Conc. HCl Test.*—Apply this test to one-fourth of the residue in a test-tube (75).

3. *Weppen's Test.*—Apply this test to one-fourth of the residue, using furfurol as suggested by Laves (76).

4. *Vitali's Test.*—Apply this test to one-fourth of the residue on a watch-glass (76).

Strychnine.—Evaporate the chloroform solution of strychnine in a dish on the water-bath. Cautiously note the taste of the residue and apply the tests designated.

1. *H_2SO_4-Dichromate Test.*—Apply this test to a small portion of the residue on a watch-glass (77).

2. *Alkaloidal Reagents.*—This alkaloid will serve to illustrate the use of general alkaloidal reagents. Put the remainder of the residue into a test-tube, add 10 c.c. of distilled water and then add cautiously enough dilute sulphuric acid to dissolve the alkaloid. Divide this solution into four equal portions in test-tubes and test with: (a) tannic acid; (b) picric acid; (c) iodine-potassium iodide; (d) potassium-mercuric iodide. Note the character of the precipitate in each case (70 and 77).

3. *Physiological Test.*—To identify an alkaloid positively, especially in medico-legal cases, the analyst should always supplement his chemical tests, whenever it is possible, by: (a) isolating the alkaloid itself in a characteristic form; (b) making a *physiological test.* Since the physiological test for strychnine is easily made and as unmistakable as a well-defined chemical test, this alkaloid has been selected by way of illustration. The instructor may perform the test on the lecture-table, or require students in small sections to do the work.

The following materials and reagents will be required:

1. Large sheets of filter paper which can be folded to make several thicknesses.

2. Large bell-jars (4) open at the top.

3. Small hypodermic syringe, the capacity of which (usually about 1 c.c.) should be determined by filling with distilled water and emptying into a small graduated cylinder.

4. Small, lively frogs (4) weighing 10-20 grams. Determine weight in a small weighed beaker with a glass cover.

5. Distilled water.

6. Acetic acid (0.1 per cent.) prepared by bringing 1 c.c. of glacial acid to a volume of 1000 c.c. with distilled water.

7. Standard strychnine solution containing 0.05 milligrams of alkaloid in 1 c.c. Weigh accurately 5 milligrams of free alkaloid and place in a 100 c.c. graduated flask. Add 50 c.c. of 0.1 per cent. acetic acid and warm on the water bath to dissolve the alkaloid. Cool and bring the volume to 100 c.c. with 0.1 per cent. acetic acid.

Procedure.—Set four bell-jars on several thicknesses of moist filter-paper. Number the jars and place a weighed frog under each. Spray the frogs occasionally with water. To make an injection, first fill the syringe with the required quantity of solution. Hold the frog firmly in the left hand and with the right hand insert the needle into the lymph heart immediately beneath the skin of the back at the root of the hind legs and slowly force in the solution. Return the frog to the jar and note the time of injection. Treat the four frogs in the following manner: I. Use as a control only; II. Inject 1 c.c. of distilled water; III. Inject 1 c.c. of 0.1 per cent. acetic acid; IV. Inject 1 c.c. of strychnine solution (0.005 per cent.). Note the result in each case, especially the length of time required for the appearance of tetanic spasms in IV (78).

Brucine.—Evaporate the alcoholic solution of brucine in a dish on the water-bath and apply the tests designated to the residue.

1. *HNO_3-$SnCl_2$ Test.*—Apply this test to a small portion of the residue on a watch-glass (79).

2. Repeat this test, following Mauch's modification (79). Note the use which can be made of strong aqueous solutions of chloral as a solvent (109)

Atropine.—Evaporate the chloroform solution of atropine in a dish on the water-bath and apply the tests designated to the residue.

1. *Vitali's Test.*—Use one-half of the residue for this test (82). What other alkaloid gives this test?

2. *Odor Test.*—Use one-half of the residue for this test (82).

3. *Physiological Test.*—This test is voluntary. The solution used may be prepared by dissolving 0.1144 gram of atropine sulphate in 100 c.c. of distilled water. A drop of this solution placed in the eye will give a distinct reaction in 15 minutes (82).

Cocaïne.—Evaporate the alcoholic solution of cocaïne in a dish on the water-bath and supply the tests designated to the residue.

1. *Physiological Test.*—Dissolve a portion of the residue on a watch-glass in a few drops of dilute hydrochloric acid and evaporate the solution to dryness on the water-bath. Dissolve the residue in a few drops of distilled water. Moisten a small strip of filter-paper with this solution and allow it to remain on the tip of the tongue until anæsthesia is observed (86).

2. *Goeldner's Test.*—Use the remainder of the residue for this test (86).

Codeïne.—Evaporate the alcoholic solution of codeïne in a dish on the water-bath and apply the tests designated to the residue.

1. *Oxidation Test.*—Apply this test to one-third of the residue on a watch-glass, using sulphuric acid containing a trace of ferric chloride as the oxidizing agent (88).

2. *Froehde's Reagent.*—Apply this test to one-third of the residue according to Mauch's method (88).

3. *Furfurol Test.*—Apply this test to one third of the residue, using the chloral hydrate method with furfurol solution instead of cane-sugar (88).

Quinine.—Evaporate the chloroform solution of quinine in a dish on the water-bath and apply the tests designated to the residue.

1. *Fluorescence Test.*—Apply this test to one-fourth of the residue in a test-tube. Repeat the test, using hydrochloric acid (92).

2. *Thalleïoquin Test.*—Apply this test to one-fourth of the residue (93).

3. *Herapathite Test.*—Apply this test to one-fourth of the residue (94).

Morphine.—Evaporate one-fourth of the aqueous solution of morphine sulphate in a watch-glass on the water-bath. For the remaining tests use the solution as received.

1. *Conc.* HNO_3 *Test.*—Apply this test to the residue obtained from one-fourth of the solution (101).

2. *Formaldehyde-*H_2SO_4 *Test.*—Apply this test to one-fourth of the solution (102).

3. *Iodic Acid Test.*—Apply this test to one-fourth of the solution in a test-tube (103).

4. *Ferric Chloride Test.*—Apply this test to one-fourth of the solution (103).

QUESTIONS.

1. Give a full description of the "Stas-Otto" procedure for the detection of alkaloids.

2. In the ether extraction of the alkaline solution, what is accomplished by using sodium hydroxide?

3. Based upon appearance and taste, what can be learned about the ether residue obtained in the above extraction?

4. Name some of the more important general alkaloidal reagents and explain their use.

5. In case the ether residue is impure, what should be done before applying the special tests?

6. What is the structural relationship of coniïne to piperidine and pyridine?

7. Name two volatile, liquid alkaloids and state why they do not appear among the volatile poisons.

8. Describe the physiological tests for strychnine, atropine and cocaïne.

9. How may morphine be distinguished from brucine?

10. Describe Vitali's test and state what alkaloids may be recognized by this test.

11. What is the structural difference between morphine and codeïne?

12. What procedure is necessary for the separation of morphine and apomorphine from the other alkaloids?

13. How may opium poisoning be differentiated from morphine poisoning?

14. Describe Mauch's use of chloral hydrate and state some advantages of this method of testing for alkaloids. Are there any disadvantages?

15. What are ptomaïnes and what is their significance in toxicological analysis?

Fifth Laboratory Period.

The work of this period deals with metallic poisons and covers pages 117 to 150 (inclusive). Except for a few changes, which facilitate the detection of metals in presence of organic matter, the methods are essentially those of inorganic qualitative analysis. Students who have had experience in detecting metals may confine themselves to certain special tests for arsenic, since toxicologists are most frequently required to detect this metalloid. But this is the time to review the principles of qualitative analysis.

The student is advised to hand in beforehand three specimen-tubes and two bottles for unknown materials to be examined for arsenic.

MATERIALS.

1. **Unknowns.**—These will be examined for arsenic.

(a) Specimen-tube containing pure calcium carbonate, or calcium carbonate mixed with 1 per cent. of white arsenic.

(b) Specimen-tube containing pure bismuth subnitrate, or bismuth subnitrate mixed with 1 per cent. of white arsenic.

(c) Specimen-tube containing pure cane-sugar, or cane-sugar mixed with 1 per cent. of potassium arsenite.

(d) Bottle containing urine, or urine mixed with 10 per cent. potassium arsenite solution (90 c.c. urine: 10 c.c. arsenite solution).

(e) Bottle containing milk, or milk mixed with 10 per cent. potassium arsenite solution (90 c.c. milk; 10 c.c. arsenite solution).

2. **Glass-Tubing.**—Hard glass-tubing having an internal diameter of 6 mm. for bulb-tubes. Instructions in making these will be given. Force a wad of filter-paper through a piece of glass 20 cm. long to remove dust. Then heat the tube in the middle and make two bulb-tubes.

3. **Absorbent Cotton.**—This is for use in making Gutzeit's test.

REAGENTS.

1. **Fusion-Mixtures.**—Two of these will be required, namely:

(a) A mixture of equal parts of dry sodium carbonate and of potassium cyanide. These substances should be finely ground and intimately mixed. This reagent is used in the bulb-tube test. *Special reagent.*

(b) A mixture of 2 parts of pure sodium nitrate and 1 part of dry sodium carbonate. These substances should be finely ground and intimately mixed. This reagent is used in destroying organic matter before applying certain arsenic tests. *Special reagent.*

2. **Sodium Nitrate.**—A chemically pure crystallized salt which must be absolutely free from arsenic. *Special reagent.*

3. **Bettendorff's Stannous Chloride.**—Prepare this reagent as directed on page 217. The ordinary solution of stannous chloride will not give satisfactory results. *Special reagent.*

4. **Arsenic-free Zinc.**—Baker and Adamson's arsenic-free zinc, either in the form of shot or granulated, is recommended. *Special reagent.*

5. **Arsenic-free HCl.**—Hydrochloric acid free from arsenic is difficult to obtain. Baker and Adamson's acid is recommended. *Special reagent.*

6. **Dil. Arsenic-free HCl.**—Dilute the concentrated acid with 5 parts of distilled water. *Special reagent.*

7. **Iodine Solution.**—See page 213.

8. **Silver Nitrate.**—Dissolve 1 part by weight of the crystallized salt in 1 part of distilled water. *Special reagent.*

9. **Copper Sheet or Wire.**—For the Reinsch test a rather heavy copper wire in pieces 3 cm. long, or sheet copper cut into strips 3 mm. wide and 3 cm. long, may be used. Before using, clean thoroughly by warming with dilute nitric acid. *Special reagent.*

EXPERIMENTAL WORK.

Arsenic.—Before examining an unknown substance for arsenic, the student should first consider the nature of the material. He should then apply appropriate tests and state in full in his written report his reasons for choosing or rejecting each test.

1. *Bulb-tube Test for Arsenic.*—Introduce a dry mixture of equal parts of substance and fusion-mixture (sodium carbonate and potassium cyanide) into a clean, dry bulb-tube. Apply heat and note the result (131). Read the following before making this test.

(a) *Test of Fusion-mixture.*—Heat a small quantity in a dish on the water-bath until it is dry. Then heat a portion by itself in a bulb-tube. If there is no mirror, the fusion-mixture is free from arsenic and may be used for tests.

(b) *Precautions.*—This test should not be made unless the material and fusion-mixture are thoroughly dry. After introducing material into the bulb-tube, carefully remove what adheres to the side of the tube. The bulb should be not more than half-full. Apply heat gradually and fuse the mixture.

(*c*) *Limitations.*—This test is applicable only to dry substances which do not contain organic matter.

2. *Bettendorff's Test for Arsenic.*—Apply this test as directed (132). Should the material be insoluble in water, it may be brought into solution with a small quantity of concentrated hydrochloric acid.

3. *Gutzeit's Test for Arsenic.*—Apply this test as directed (132). Set the test-tube under the hood while the reaction is going on.

4. *Reinsch's Test for Arsenic.*—This test is applicable even to liquids containing organic matter (urine, etc.). Strongly acidify the liquid in a test-tube with concentrated hydrochloric acid. To make sure that the acid is pure, run a blank and note if a deposit appears on the copper. If the test is negative, add to the acid liquid bright copper wire or strips. Heat the test-tube in a beaker of boiling water. Arsenic will form a gray coating on copper. Mercury, antimony, bismuth, etc., under the same conditions will give similar coatings. When the action has ceased, wash the copper carefully and dry it on filter-paper. Distinguish arsenic from other metals by heating the copper in a hard glass-tube. This tube should be open at both ends, about 30 cm. long and perfectly clean. Place the copper 5 cm. from the lower end. Incline the tube, regulate the air-

current with the fore-finger over the upper end and heat the copper. Metallic arsenic will be oxidized and deposited as a sublimate of octahedral crystals easily visible with a hand-lens.

QUESTIONS.

1. Describe the method of destroying tissues, preparatory to tests for metallic poisons, in which hydrochloric acid and potassium chlorate are used. Are there any objections to this method? What is an alternative procedure?

2. When hydrogen sulphide produces a precipitate in the solution freed from organic matter, what conclusions can be drawn?

3. How would you prepare arsenic-free hydrogen sulphide?

4. At what point in the analysis is Meyer's fusion-method used and what does it accomplish?

5. Describe the Fresenius-von Babo test for arsenic and make a sketch of the apparatus.

6. Give a detailed description of the Berzelius-Marsh test for arsenic and make a sketch of the apparatus.

7. State all the differences and resemblances shown by the spots and mirrors given by arsenic and antimony.

8. Describe in full the method of testing for arsenic biologically. What are the advantages and disadvantages of this test?

9. What is meant by "normal arsenic"?

10. What can you say about the elimination of lead, mercury and tin?

11. In case of poisoning by antimony, copper or zinc, where would you look for these metals?

12. What can you say about the physiological action of poisonous quantities of chromium and silver?

13. Describe Bettendorff's, Gutzeit's and Reinsch's test for arsenic.

SIXTH LABORATORY PERIOD.

The work of this period deals with acids, alkalies and substances having no place in the groups already studied. A description of the methods and tests is given on pages 151 to 167 (inclusive).

The student is advised to hand in beforehand three bottles and two specimen tubes for unknown solutions and solids which will be examined as directed.

MATERIALS.

1. **Unknowns.**—These will be made up as follows:

(a) Bottle containing an aqueous solution of free acid or acids. To prepare this solution, use 2 per cent. solutions of mineral or organic acids. Having applied the selected tests, the student will answer these questions. Is a free acid present? Is the acid mineral or organic? What acid or acids do you find? State the tests used and give reasons in full for your conclusions.

(b) Bottle containing an aqueous solution of ammonium, sodium or potassium as hydroxide or carbonate. To prepare this solution, use 2 per cent. solutions of the alkaline hydroxides or carbonates. In your report upon this solution, follow the plan given in (a).

(c) Bottle containing an aqueous solution of potassium chlorate or of oxalic acid, one or both. To prepare this solution, use 2 per cent. solutions of these substances. In your report upon this solution, follow the plan given in (a).

(d) Specimen-tube containing powdered milk-sugar, or milk-sugar mixed with 2 per cent. of santonin. The student should consider the possible interference of milk-sugar with santonin tests and devise a method of obviating this difficulty, if it exists.

(e) Specimen-tube containing one of three things, namely: 1. sulphonal; 2. a mixture of sulphonal and some other substances such as acetanilide, phenacetine, etc.; 3. a pure substance or mixture which does not contain sulphonal Is sulphonal present? Is it pure or mixed with another substance? With what substance do you think it is mixed?

REAGENTS.

1. **Alcoholic Methyl Violet.**—Dissolve 1 part by weight of methyl violet in 100 c.c. of strong alcohol. *Special reagent.*

2. **Congo Paper.**—Prepare a saturated, aqueous solution of Congo red. Dip strips of filter-paper in this solution, dry the strips in air and cut into small pieces for use. *Special reagent.*

3. **Günzburg's Reagent.**—Prepare this reagent as directed on page 216, but keep the two solutions separate, mixing them volume for volume as needed. *Special reagent.*

4. **Manganese Dioxide.**—Commercial black oxide of manganese. *End-desk.*

5. **Diphenyl-amine Sulphate.**—Dissolve 1 part by weight of diphenyl-amine in 5 c.c. of dilute sulphuric acid and bring the volume to 100 c.c. with distilled water. *Special reagent.*

6. **Brucine Sulphate.**—Dissolve 1 part by weight of brucine in 5 c.c. of dilute sulphuric acid and bring the volume to 100 c.c. *Special reagent.*

7. **Cane-Sugar.**—Crystallized cane-sugar, the so-called "rock-candy." *Special reagent.*

8. **Phenol-phthaleïn.**—Dissolve 1 part by weight of phenol-phthaleïn in 100 c.c. of strong alcohol and dilute this solution with 50 c.c. of distilled water. *Special reagent.*

9. **Barium Chloride.**—Dissolve 1 part by weight of the crystallized salt in 10 parts of water. *End-desk.*

10. **De Konink's Reagent.**—Prepare this reagent as directed in the foot-note on page 158. *Special reagent.*

11. **Nessler's Reagent.**—See page 217.

12. **Indigo Solution.**—Pour 5 parts of fuming sulphuric acid into a beaker, place the latter in a dish of water to keep it cool, and stir into the acid, little by little, 1 part of finely powdered indigo. Let this mixture stand 48 hours; then pour it into 20 times its own volume of water; filter the mixture and use the filtrate. *Special reagent.*

13. **Sulphurous Acid.**—Use the commercial acid which is a saturated aqueous solution of sulphur dioxide. *Special reagent.*

14. **Silver Nitrate.**—See page 221.

15. **Dilute Nitric Acid.**—See page 222.

16. **Conc. Hydrochloric Acid.**—See page 228.

17. **Potassium Iodide.**—Dissolve 1 part by weight of the crystallized salt in 10 parts of water. *Special reagent.*

18. **Chloroform.**—See page 228.

19. **Ammonium Hydroxide.**—See page 222.

20. **Calcium Sulphate.**—A saturated aqueous solution of calcium sulphate. *End-desk.*

21. **Acetic Acid.**—See page 229.

22. **Alcoholic Potassium Hydroxide.**—See page 227.

23. **Conc. Sulphuric Acid.**—See page 228.

24. **Ferric Chloride.**—See page 221.

25. **Alcoholic Furfurol.**—Dissolve 2 c.c. of furfurol in 100 c.c. of strong alcohol. *Special reagent.*

26. **Potassium Cyanide.**—The dry salt, a very small quantity of which should be issued by the instructor to each student. *Special reagent.*

27. **Dilute Hydrochloric Acid.**—See page 221.

28. **Wood Charcoal.**—Powdered wood charcoal. *Special reagent.*

EXPERIMENTAL WORK.

Mineral Acids.—First determine by the following general tests whether or not a mineral acid is present.

1. *Methyl Violet Test.*—Use 5 c.c. of the solution for this test (151).

2. *Congo Paper Test.*—Having applied this test to the unknown solution, dilute a small quantity of acetic acid with water and repeat the test (151).

3. *Günzburg's Test.*—Use 5 c.c. of the solution for this test (151).

If a mineral acid is found to be present, make the following special tests to determine the particular acid or acids.

1. *Chlorine Test.*—Evaporate 10 c.c. of the solution to 2 c.c. and apply this test as directed, introducing a small quantity of manganese dioxide through a paper tube in order not to soil the walls of the test-tube (152).

2. *Diphenyl-amine-H_2SO_4 Test.*—Use 5 c.c. of the solution for this test (154).

3. *Brucine-H_2SO_4 Test.*—Use 5 c.c. of the solution for this test (154).

4. *Sugar Test.*—Use 5 c.c. of the solution for this test (156).

Alkalies.—First determine by means of turmeric paper or phenol-phthaleïn solution whether or not an alkali is present.

If an alkali is found to be present, put about one-half of the solution into a small flask and distil nearly to dryness. Keep both distillate and residue and test each separately.

1. *Distillate.*—Test for the presence of ammonia either with copper sulphate solution or with a few drops of Nessler's solution. In making the latter test, it is advisable to dilute the distillate considerably.

2. *Residue.*—Dissolve the residue in 20 c.c. of water and divide the solution into two equal portions. Use one-half to determine whether hydroxide or carbonate is present by means of the method with barium chloride (157). Test the remainder of the solution with de Konink's reagent (158).

Potassium Chlorate.—Apply the following tests for the detection of this substance.

1. *Indigo Test.*—Use 5 c.c. of the solution for this test (159).

2. *Silver Nitrate Test.*—Use 5 c.c. of the solution for this test (159).

3. *Free Chlorine Test.*—Use 5 c.c. of the solution for this test (159).

Oxalic Acid.—Apply the following test for the detection of this substance.

1. *Microscopic Examination.*—Render 10 c.c. of the solution alkaline with ammonium hydroxide solution, add calcium sulphate solution and proceed as directed (162).

Santonin.—By appropriate means isolate santonin from the milk-sugar with which it is mixed and make the following tests.

1. *Alcoholic KOH Test.*—Apply this test to an alcoholic solution of a portion of the santonin (164).

2. *H_2SO_4-$FeCl_3$ Test.*—Apply this test to dry santonin (165).

3. *Furfurol-H_2SO_4 Test.*—Apply this test to an alcoholic solution of santonin (165).

Sulphonal.—Use a capillary tube and determine the melting-point of the substance in the usual way, bearing in mind that a mixture has a lower melting-point than that of either constituent. If this test is not sufficient, apply the following tests.

1. *Vulpius' Test.*—Apply this test as directed (166).

2. *Reduction Test.*—Apply this test as directed (166).

QUESTIONS.

1. In an examination for the presence of free mineral acids, what objection can be raised against testing for sulphuric acid with barium chloride, or for hydrochloric acid with silver nitrate?

2. How do aqueous solutions of hydrochloric acid, or of nitric acid, behave when distilled?

3. Why is the quantitative determination of hydrochloric acid essential in case of poisoning by this acid?

4. What are some of the physical signs indicating poisoning by mineral acids?

5. How is it possible to differentiate alkaline hydroxides from alkaline carbonates?

6. What is the physiological action of potassium chlorate?

7. What effect has putrefaction upon potassium chlorate? How might this be explained?

8. What organs should be examined in case of poisoning by oxalic acid? Why should this substance be determined quantitatively?

9. What is the fate of santonin in the organism?

10. What effect have sulphonal and trional upon the urine?

Seventh Laboratory Period.

The work of this period deals with the detection of blood and covers pages 167 to 181 (inclusive). Having made the more important tests for blood, the student should be required to examine unknown materials.

Materials.

1. **Defibrinated Ox-Blood.**—Rapid agitation of freshly drawn ox-blood with the hands will cause the fibrin to separate in fine threads. If necessary, the entire mass may then be thrown upon cheese-cloth and the defibrinated blood drained from the fibrin. Supply each student with 10 c.c. of this blood.

2. **Carbon Monoxide-Blood.**—This may be prepared by passing through defibrinated blood either carbon monoxide gas or ordinary illuminating gas. Supply each student with 10 c.c. of this blood.

Blood Stains.—Stain cloth with blood and also with other substances having the appearance of blood. Students should be supplied with pieces of such cloth for examination.

REAGENTS.

1. **Sodium Hydroxide.**—See page 221.

2. **Potassium Ferrocyanide.**—Dissolve 1 part by weight of crystallized potassium ferrocyanide in 5 parts of water. *Special reagent.*

3. **Acetic Acid.**—See page 229.

4. **Tannic Acid.**—Dissolve 1 part by weight of tannic acid in 100 parts of water. *Special reagent.*

5. **Stokes' Reagent.**—Dissolve 2 parts by weight of ammonium ferrous sulphate ($FeSO_4.(H_4N)_2SO_4.6H_2O$) and 3 parts of tartaric acid in 50 c.c. of water. Render this solution alkaline with ammonium hydroxide and then bring the volume to 100 c.c. with water. This solution should be freshly prepared. *Special reagent.*

6. **Formaldehyde.**—Use 40 per cent. solution of formaldehyde gas in water. *Special reagent.*

7. **Glacial Acetic Acid.**—Use the commercial article which should contain at least 98 per cent. of the absolute acid. *Special reagent.*

8. **Sodium Chloride.**—Pure, crystallized sodium chloride. *Special reagent.*

9. **Sodium Iodide.**—Dissolve 1 part by weight of crystallized sodium iodide in 500 parts of water. *Special reagent.*

10. **Ozonized Turpentine.** — Should old turpentine, which has been exposed to air for a long time, not be available, it is possible to prepare a satisfactory reagent by using an air-pump to draw a current of air through new turpentine for 12 to 24 hours. *Special reagent.*

11. **Guaiac Tincture.**—Prepare this reagent as directed at the bottom of page 17. *Special reagent.*

12. **Chloral Hydrate.**—Dissolve 70 grams of crystallized

chloral hydrate in the smallest possible quantity of water and bring the final volume to 100 c.c. *Special reagent.*

13. **Chloral Hydrate-Guaiac.**—Extract 10 grams of powdered gum guaiac with 100 c.c. of 70 per cent. aqueous choral hydrate solution prepared as above. *Special reagent.*

14. **Hünefeld's Reagent.**—Prepare this reagent as directed on page 216. *Special reagent.*

15. **Hydrogen Peroxide.**—Use the commercial, ten-volume solution of hydrogen peroxide. If there is doubt as to its efficiency, test for hydrogen peroxide with dilute hydrochloric acid, potassium dichromate and ether. *Special reagent.*

16. **Chloral Hydrate-Aloïn.** — Extract 10 grams of aloïn with 100 c.c. of 70 per cent. aqueous chloral hydrate solution prepared as above. *Special reagent.*

Experimental Work.

Carbon Monoxide in Blood.—In the following tests use defibrinated ox-blood through which carbon monoxide has been passed. If the blood has not been diluted, add the necessary quantity of water in case the test calls for dilution (167).

1. *Sodium Hydroxide Test.*—Apply this test as directed both to normal blood and to carbon monoxide blood (168).

2. **Potassium Ferrocyanide Test.**—Apply this test as directed both to normal blood and to carbon monoxide blood (168).

3. *Tannin Test.*—Apply this test as directed both to normal blood and to carbon monoxide blood (168).

4. *Spectroscopic Examination.*—The instructor will show to small sections of the class the following points in the spectroscopic examination of blood for carbon monoxide (169).

(a) Dilute 1 part of normal blood with 100 c.c. of water and note the absorption-bands of oxy-haemoglobin. Reduce another portion of diluted blood with Stokes' solution (alkaline ferrous sulphate and tartrate) and note replacement of the two oxy-haemoglobin bands by the haemoglobin band.

(b) Dilute 1 part of carbon monoxide blood with 100 c.c. of water and note the two absorption-bands of carboxy-haemoglobin. How do they differ from those of oxy-haemoglobin? Reduce another portion of the same blood with Stokes' solution and note that the absorption-bands remain unchanged.

(c) Apply Tollens' procedure with formaldehyde to both kinds of diluted blood. Add 4 drops of 40 per cent. formaldehyde solution before showing the effect of a reducing agent.

Blood Stains.—Pieces of cloth stained with human and with animal blood, or with dye-stuffs resembling blood, will be examined. The blood should be removed from the fabric as directed and the following tests applied (171).

1. *Haemin Crystals.*—Apply this test as directed. Repeat according to Strzyzowski's modification (172).
2. *Schönbein-Van Deen Test.*—Apply this test as directed (175).
3. *Vitali's Procedure.*—Apply this test as directed (177).
4. *Schaer's Procedure.*—Apply this test as directed (178).
5. *Schaer's Aloïn Test.*—Apply this test as directed (178).

QUESTIONS.

1. Describe three tests which make it possible to recognize the presence of carbon monoxide in blood.
2. In how many ways can you differentiate oxy-haemoglobin from carboxy-haemoglobin with the spectroscope? Describe each way.
3. Describe in full your procedure, if you were asked to examine a stain upon cloth for blood.

4. Describe the detection of blood by the Schönbein-Van Deen test and by the aloïn test.

5. Describe in full the procedure necessary for the biological test for blood.

6. What should be the order of procedure in differentiating human from animal blood?

INDEX.

Absorption-spectra, 170
Acetanilide, 59, 163
  solution of, 233
Acetone, 40
  detection in urine, 42
  solution of, 227
Acid, acetic, reagent, 229
  acetic glacial, reagent, 257
  carbolic, 21
   reagent, 234, 257
   solution of, 219
   with aniline, 28
  chromic, reagent, 235
  formic, from chloral hydrate, 32
  hydrochloric, conc. arsenic-free, reagent, 248
   conc. reagent, 228
   detection, 152
   dilute, reagent, 221
  hydrocyanic, determination, 20
   solution of, 219
   with potassium ferrocyanide, 20
  meconic, 106
  nitric, conc., reagent, 220
   detection, 154
   dilute, reagent, 222
   fuming, reagent, 240
  oxalic, 161
  phospho-molybdic, reagent, 214
  phosphorous, detection, 10
  phospho-tungstic, reagent, 215
  picric, 57
   reagent, 215, 222
   solution of, 233
  salicylic, 66
   solution of, 234
  selenious, reagent, 108
  sulphuric, conc., reagent, 228
   detection, 156
   dilute, reagent, 222
  sulphurous, reagent, 253
  tannic, reagent, 215, 234, 257
  tartaric, reagent, 221
Acids, mineral, 151
Aconitine, estimation in aconite root, 210
Alcohol, ethyl, absolute, reagent, 241
   detection, 37
   solution of, 226
Alkalies, detection, 157
Alkaloids, Stas-Otto method, 49
Aloïn, chloral hydrate solution, reagent, 258
Ammonia, detection, 158
Ammonium ferrous sulphate, reagent, 221, 257
  hydroxide conc., reagent, 229
   dilute, reagent, 222
  molybdate, reagent, 220
  sulphide, reagent, 222
   yellow, reagent, 221
Aniline, 35, 74
  hydrochloride, reagent, 223
  solution of, 226
Antimony, detection, 134
  fate, distribution and elimination, 146
  mirror and spot, 128
Antipyrine, 62, 94
  solution of, 234
Apomorphine, 97
Arsenic, Berzelius-Marsh test, 125
  Bettendorff's test, 132
  biological test, 142
  detection, 124
  distinction from antimony, 128
  fate, distribution and elimination, 145
  Fresenius-von Babo test, 130
  Gutzeit's test, 132
  mirror and spot, 127
  normal occurrence in body, 146
  Reinsch test, 249
Atropine, 80
  estimation in extracts, 207, 208
  solution of, 239

Autenrieth-Hinsberg phenacetine test, 61

Barium, 141
  chloride, reagent, 252
Benzaldehyde, 45
  alcoholic, reagent, 234
  ortho-nitro, reagent, 229
  reagent, 223
Benzoyl chloride, reagent, 228
Berthellot's alcohol test, 38
Berzelius-Marsh apparatus, 126
Bettendorff's arsenic test, 132
Biological arsenic test, 142
Biological blood test, 179
Bismuth, detection, 136
Bismuth-potassium iodide, reagent, 214
Bitter almond water, 45
Blondlot apparatus, 11
Blood, carbon monoxide, 256
Blood-stains, detection, 171
Bouchardat's reagent, 213
Bromine water, reagent, 222
Brucine, 78
  estimation in nux vomica, 203
  solution of, 239
  sulphate, reagent, 252

Cadmium, detection, 137
Cadmium-potassium iodide, reagent, 213
Caffeïne, 64, 94
  estimation in coffee, tea and cola-nuts, 185
  solution of, 234
Calcium hypochlorite, reagent, 222
  sulphate, reagent, 253
Cane-sugar, reagent, 252
Cantharidin, estimation in Spanish flies, 182
Carbolic acid, 21
Carbon disulphide, detection, 42
    estimation in air, 43
    reagent, 228
    solution of, 227
Carbon monoxide in blood, detection, 167
Carboxy-hæmoglobin, absorption-spectrum, 170
Cephaëline, estimation in ipecac, 193
Charcoal, wood, reagent, 253
Chloral hydrate, 32
    solution of, 226
    use as a solvent, 109, 240, 257
Chlorine water, reagent, 220
Chloroform, detection, 28
  estimation in cadavers, 30
  reagent, 228
  solution of, 219
Chromium, 139
  fate, distribution and elimination, 147
Cinchona alkaloids, estimation in bark, 183
Cocaïne, 84
  solution of, 239
Codeïne, 87
  solution of, 239
Colchicin, 56
  solution of, 233
Congo paper, preparation, 252
Coniïne, 71
Copper, 123, 133, 136, 137
  fate, distribution and elimination, 147
  reagent, 248
  sulphate, reagent, 221, 229

De Konink's reagent, preparation, 252
Digitaline, 108
Digitalis glucosides, 108
Digitonin, 108
Digitoxin, 108
Diphenyl-amine sulphate, reagent, 252
Distillation of volatile poisons, 14
Dragendorff's reagent, 214

Emetine (see cephaëline)
Erdmann's reagent, preparation, 215
Ether, solvent, 228
Extract of belladonna, estimation, 207
  of hyoscyamus, estimation, 208
  of nux vomica, estimation, 208
Extraction method of Stas-Otto, 49

Fehling's solution, preparation, 215
Ferric chloride, neutral, reagent, 241
    reagent, 221
Ferrous sulphate, reagent, 221
Formaldehyde, reagent, 257
  sulphuric acid, morphine test, 102

Formaldehyde, sulphuric acid, reagent, 216, 241
Formic acid, from chloral hydrate, 32
Fresenius-von Babo apparatus, 131
Froehde's reagent, preparation, 215
Furfurol, reagent, 240, 253
Fusion mixtures, reagents, 247

Gautier, arsenic normally in body, 146
Glucose, reagent, 234
Goëldner's cocaïne test, 86
Gold chloride, reagent, 213
Guaiac, chloral hydrate solution, reagent, 258
Guaiac-copper paper, 17
Günzburg's reagent, 216
Gutzeit's arsenic test, 132

Haematoporphyrin, absorption-spectrum, 170
    detection in urine, 167
Haemin crystals, 170
        Strzyzowski's method, 172
Haemochromogen, absorption-spectrum, 175
Haemoglobin, reduced, absorption-spectrum, 170
Herapathite quinine test, 94
Homatropine, 82
Human blood, detection, 179
Hünefeld's solution, reagent, 216
Husemann's reaction, 97, 101
Hydrastine, estimation in fluid extract of hydrastis, 196
Hydrastinine, 197
Hydrochloric acid, 152
Hydrocyanic acid, 16
Hydrogen peroxide, reagent, 258
    sulphide, arsenic-free, 120
        water, reagent, 221
Hyoscyamine, 81

Indigo solution, reagent, 253
Indophenol reaction, 59, 62
Iodic acid, reagent, 217
        preliminary morphine test, 98
Iodine, alcoholic solution, reagent, 241
    potassium iodide solution, reagent, 213
Iodoform, detection, 33
    solution of, 226

Ipecac, estimation of alkaloid in, 192

Koenig's quinine formula, 92
Koppeschaar-Beckurt's, estimation of carbolic acid, 26
Kossler-Penny, estimation of carbolic acid, 27

Langley's picrotoxin test, 55
Lead acetate, reagent, 221
    detection, 136, 141
    fate, distribution and elimination, 146
Legal's acetone test, 41
Lieben's reaction, 38, 40
Liebig's distillation apparatus, 15
Ludwig, chloroform in cadavers, 30

Magnesia mixture, preparation, 6, 217
Magnesium oxide, reagent, 227
Manganese dioxide, reagent, 252
Marmé's reagent, 213
Marsh apparatus, 126
Mayer's reagent, 213
Meconic acid, 106
Meconine, 107
Melzer's carbolic acid test, 25
Melzer's picrotoxin test, 55
Mercuric chloride, reagent, 213, 227
        and volatile arsenic compound, 143
    cyanide, detection, 21
Mercuric-potassium iodide, reagent, 213
Mercury, detection, 135
    detection in urine, 148
    fate, distribution and elimination, 148
Metallic poisons, detection, 117
Metals, distribution in body and elimination, 145
Methaemoglobin, absorption-spectrum, 175
Methyl violet, reagent, 252
Meyer's separation of arsenic, antimony and tin, 123
Millon's reagent, preparation, 217
Mineral acids, detection and determination, 151
Mitscherlich apparatus for phosphorus, 5
Morphine, detection, 98

Morphine, estimation in opium and its preparations, 198, 199
  sulphate, solution of, 240

Naphthol, alpha, reagent, 223
Narceïne, 104
Narcotine, 89
Nessler's reagent, preparation, 217
Nicotine, 73
  estimation in tobacco, 194
Nitric acid, 154
Nitrobenzene, 34
  solution of, 226
Non-volatile, organic poisons, 49
    apparatus for extraction, 50

Opium, detection, 106
Oxalic acid, 161
Ox-blood, defibrinated, preparation, 256
Oxyhaemoglobin, absorption-spectrum, 170

Pellagri's reaction, 89, 97, 102
Penzoldt's acetone test, 41
Phenacetine, 61, 163
  solution of, 233
Phenol, 21
Phenol-phthaleïn, reagent, 252
Phenyl-isocyanide reaction, 29, 34, 37, 60
Phospho-molybdic acid, reagent, 214
Phosphorous acid, detection, 10
Phosphorus, Blondlot-Dussard method, 10
Phosphorus, determination, 8
  Hilger-Nattermann method, 9
  Mitscherlich method, 4
  solution of, 219
Phospho-tungstic acid, reagent, 215
Physiological test for atropine, 82
    for cantharidin, 183
    for cocaïne, 86
    for physostigmine, 84
    for strychnine, 78, 242
Physostigmine, 83
Picric acid, 57
    reagent, 215, 222
Picrotoxin, 54
  detection in beer, 56
  solution of, 233
Platinum chloride, reagent, 213
Pomegranate bark, estimation of alkaloids in, 210
Potassium bismuthous iodide, reagent, 214
  cadmium iodide, reagent, 213
  chlorate, detection, 159
    to destroy organic matter, 117
  chromate, reagent, 228
  cyanide, reagent, 234, 253
  dichromate, reagent, 240
  ferrocyanide, reagent, 257
  hydroxide, alcoholic, reagent, 227
    reagent, 221
    saturated alcoholic, reagent, 229
    solid, reagent, 228
  iodide, reagent, 253
  mercuric iodide, reagent, 213
  nitrate, reagent, 234
Potassium nitrite, reagent, 221
Ptomaïnes, 111
Pyramidone, 95

Quinine, 91
  solution of, 240

Reagents, preparation, 212, 220, 227, 234, 240, 247, 252, 257
Resorcinol, reagent, 223, 241
Reynold's acetone test, 41
Roussin's crystals, 74

Salicylic acid, 66
    solution of, 234
Santonin, detection, 164
  estimation in wormseed, 200
Scheibler's reagent, 215
Schaer's aloïn blood test, 178
  guaiac blood test, 178
Schönbein's hydrocyanic acid test, 17
  blood test, 175
Selenious acid, reagent for alkaloids, 108
Selenium, biological arsenic test, 143
Silver, detection, 141
  determination in organs, 149
  fate, distribution and elimination, 149
  nitrate, reagent, 221, 248
Sodium acetate, anhydrous, reagent, 228
  chloride, reagent, 257

Sodium hydroxide, reagent, 221
  hyposulphite, reagent, 227
  iodide, reagent, 257
  nitrate, reagent, 248
  nitrite, reagent, 221
  nitro-prusside, reagent, 229
  phenolate, reagent, 227
Sonnenschein's reagent, 214
Soxhlet extraction apparatus, 189
Stannous chloride, reagent, 217, 240
Stas-Otto extraction method, 51
Stokes' solution, preparation, 257
Strychnine, detection, 76
  estimation in nux vomica, 203
  physiological test, 78, 242
  solution of, 239
Sulphonal, 165
  detection in urine, 166
Sulphuric acid, 156
Synopsis of Group I, 46
  of Group II, 112
  of Group III, 141

Tannic acid, reagent, 215, 234, 257
Teichmann's crystals, 170
Tellurium, biological arsenic test, 143
Thalleïoquin reaction for quinine, 93
Theïne (see caffeïne)
Theobromine, estimation in cacao-seeds, 205
Thread, in detecting picric acid, 234
Tin, detection, 134
  fate, distribution and elimination, 150
  reagent, 228
Tincture of nux vomica, estimation of alkaloid in, 209
Trional, 166
Turpentine, ozonized, reagent, 257

Van Deen's blood test, 175
Veratrine, 74
  solution of, 239
Vitali's alcohol test, 39
  atropine test, 82
  blood test, 177
  veratrine test, 76
Volatile poisons, 3
Vongerichten's morphine formula, 101
Vortmann's nitro-prusside test, 19
Vulpius' sulphonal test, 166

Wangerin's apomorphine test, 98
Weppen's reaction, 76

Zinc, arsenic-free, reagent, 248
  detection, 137
  fate, distribution and elimination, 149

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